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Morphology of Palatal Rugae in Various Sagittal Skeletal Malocclusions in Kerala Population- A Retrospective Study

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Abstract

The present study was designed evaluate the structural morphology of palatal rugae in Kerala orthodontic subjects with varying Sagittal Skeletal Malocclusions. Pretreatment maxillary casts of 105 patients were analyzed for rugae patterns qualitatively and quantitatively using modified Thomas and Kotze classification. The data were statistically analyzed. According to the Fishers exact test, the most common type of rugae pattern in all the groups (Class I, Class II, and Class III) were observed to be wavy followed by curved. Skeletal Class III had the highest number of straight rugae 71.4% among the three groups which was statistically significant. Direction of rugal alignment was horizontal and droplet-shaped incisive papilla was identified among different skeletal dysplasia groups.Palatal ruage may serve as valuable indicators for population identification and help in facial reconstructions through their association with varying skeletal malocclusions.

Keywords: Palatal Rugae Pattern, Skeletal Malocclusion, Dental Casts, Forensic Dentistry

Introduction

The irregular asymmetrical palatine folds on the anterior third of the palate are called as palatal rugae. They possess unique individualistic morphologic characteristics that are helpful in population identification and are stable over time.1, 2

In a county like India where usable dental anthropological data is sparse palatal rugae could be valuable for forensic identification due to racial specificity, facilitating population identification and post-mortem identification.

Rugae are classified based on their length, shape, direction of alignment, unification or branching number distributed on either side of the median palatal raphae. The plica palatinae are thus assessed qualitatively and quantitatively based on these parameters.

Studies have shown the uniqueness of the rugae when assessed in various populations and may be helpful in individual identification and population identification.3- 7

Stability of palatal rugae, pre and post orthodontic treatment have made them reliable markers of identification. Studies have also attempted to establish the relation of palatal rugae pattern and various dental malocclusions, thereby allowing for early intervention and prevention of dento-skeletal issues.8, 9 A study was conducted to investigate morphological structure of palatal rugae in Turkish orthodontic subjects with various sagittal skeletal malocclusions, concluded the uniqueness of rugae morphology in relation to skeletal dysplasia.10

No study has been conducted on individuals with various sagittal skeletal malocclusions in Indian population with varying ethnicities. The purpose of this study was to evaluate the morphological structure...
of palatal rugae in Kerala orthodontic subjects with different sagittal skeletal malocclusions.

The objective of the present study was to evaluate and compare the structural morphology (number, pattern, direction of alignment) of palatal rugae and shape of the incisive papilla in Kerala orthodontic subjects with varying sagittal skeletal malocclusions i.e. Skeletal Class I, Skeletal Class II and Skeletal Class III.

**Material and Method**

The study was designed to be a retrospective study. Pretreatment records i.e lateral cephalograms and maxillary casts of 105 subjects age ranging 18-25 years, were procured from the archives of the department. Records of patients with any congenital deformity or history of palatal surgery and previous history of orthodontic treatment were excluded. The maxillary models whose palatal rugae were clearly visible were selected.

**Methodology**

The subjects were grouped into Class I, Class II, Class III (n=35 each) according to the ANB angle measured on lateral cephalometric radiographs. (Class I ANB angle 0º to 4º, Class II ANB angle >4º and Class III ANB angle <0º).

The maxillary casts were numbered. A midline was drawn coinciding with that of the mid palatine raphae extending from the incisive papillae to the posterior most extent of the rugae dividing the palate into right and left halves. The outlines of the rugae were traced using graphite pencil under adequate illumination. The rugae were then measured and categorized as primary, secondary and tertiary rugae and assessed for different patterns. Modified Thomas and Kotze\(^{11}\) method was used for quantitative and quantitative assessment. The number, pattern, direction of rugae alignment and shape of the incisive papilla were assessed among all the groups.

**Statistical Analysis**

All data were analysed using IBM SPSS software VERSION 22.0. The Kruskal–Wallis test was used to compare the number of primary palatal rugae (PPR) among the groups. Fishers exact test was used to compare the pattern of rugae between the various skeletal malocclusions on both sides of the palate and the direction of rugae among the groups. Chi square test was used to compare the shape of the incisive papilla among the groups. P values of less than 0.05 were considered statistically significant.

**Results**

Kruskal- Wallis test analyzed the primary palatal rugae (PPR) quantitatively and compared among the groups on the right and left side of the palate. As regards to the number of PPR on the left and right sides, there was maximum number of primary palatal rugae on the right side of subjects with Skeletal Class II (mean 3.86right and 3.80 left) and Skeletal Class III (mean 4.17 right and 3.97 left) in comparison with Skeletal I (mean 3.54 right and 3.60 left) with the highest number of primary rugae in Skeletal Class III on the right side among the groups, which was statistically significant (p value 0.003) (Table 1).

**Table 1: Kruskal Wallis test showing the number of primary palatal rugae (PPR) among the groups**

<table>
<thead>
<tr>
<th>Primary</th>
<th>Class</th>
<th>N</th>
<th>Mean (SD)</th>
<th>Range</th>
<th>Median (Q1-Q3)</th>
<th>Chi square value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right</td>
<td>Class 1</td>
<td>35</td>
<td>3.54 (0.61)</td>
<td>2 - 5</td>
<td>4(3 - 4)</td>
<td>11.90</td>
<td>0.003*</td>
</tr>
<tr>
<td></td>
<td>Class 2</td>
<td>35</td>
<td>3.86 (0.65)</td>
<td>3 - 5</td>
<td>4(3 - 4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Class 3</td>
<td>35</td>
<td>4.17 (0.79)</td>
<td>3 - 6</td>
<td>4(4 - 5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>Class 1</td>
<td>35</td>
<td>3.60 (0.55)</td>
<td>3 - 5</td>
<td>4(3 - 4)</td>
<td>4.33</td>
<td>0.12(NS)</td>
</tr>
<tr>
<td></td>
<td>Class 2</td>
<td>35</td>
<td>3.80 (0.58)</td>
<td>3 - 5</td>
<td>4(3 - 4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Class 3</td>
<td>35</td>
<td>3.97 (0.89)</td>
<td>2 - 6</td>
<td>4(3 - 5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N=Samples In Each Group*p<0.05 statistically significant,
Wavy and curved types were the most common types of rugae pattern in all skeletal dysplasia groups. On the right side (Class I, 93% wavy and 31.4% curved, Class II, 91% wavy and 51.4% curved, Class III, 80% wavy and 65.7% curved). Followed by straight and unification pattern. Skeletal Class III had the highest number of straight rugae 71.4% among the three groups which was statistically significant (p value 0.00) (Figure 1). On the left side of the palate Wavy pattern was the most common pattern in all malocclusion groups (Class I, 94.3% wavy, Class II 94.3% wavy, Class III, 77.1%), but this was not statistically significant. Followed by curved, straight and unification pattern. Skeletal Class III had the highest number of straight rugae 62.9% among the three groups which was statistically significant (p value 0.003). (Figure 2)

**Figure 1:** Graph showing the pattern of rugae on right side among the groups.

**Figure 2:** Graph showing the pattern of rugae on left side between the groups.

Fischer’s exact test compared the direction of rugae among the groups. Direction in all groups was horizontal (54.3% Class I, 85.7% Class II, 68.6% Class III). Irregularly directed rugae were seen highest in Skeletal Class I group (40.0%) as compared to the other groups and this was statistically significant (p value 0.003). (Figure 3).
Figure 3: Fisher’s Exact Test showing the direction of rugae alignment in different skeletal malocclusions among the groups

Droplet-shaped incisive papilla was identified to be predominant among different skeletal malocclusion groups. (Table 2).

Table 2:

<table>
<thead>
<tr>
<th>Shape of incisal papilla</th>
<th>Malocclusion</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Class 1</td>
<td>Class 2</td>
</tr>
<tr>
<td>Cylindrical</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>31.4%</td>
<td>20.0%</td>
</tr>
<tr>
<td>Droplet</td>
<td>24</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>68.6%</td>
<td>80.0%</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Chi square test

Chi square value = 1.33(2), p-value = 0.52(NS)

* p<0.05 statistically significant, p>0.05 Non significant, NS

Table 2: Chi Square Test showing the shape of the incisive papilla among the groups

Discussion

Palatal rugae with their individual uniqueness have aided in forensic identification and population identification and have imposed their importance in cases where digit biometrics are unavailable. The shape of the palatal rugae seems to remain constant throughout life.\(^\text{12}\) Palatal rugae facilitate population identification as they possess identical characteristics amongst certain racial groups.\(^\text{13}\) Like fingerprints the palatal rugae do not alter during the course of life with only increase in length due to normal growth.\(^\text{14}\)

Even though examination of the palatal rugae is relatively a subjective process, it can be easily recorded sans any complex armamentarium. Palatal rugae are unique in each individual similarly, in this present study, each subject’s palatal rugae were found to be unique.\(^\text{15-18}\)

Some authors suggest that palatal rugae remain unchanged as of 12\(^\text{th}\) weeks of intrauterine life; whereas other authors suggest that palatal rugae may change quantitatively with increasing age but retain their general configuration.\(^\text{19, 20}\)
In the present study we only assessed the number and shape of the palatal rugae among different skeletal malocclusion groups and no time related evaluation of the rugae was undertaken.

The number of PPR ranged from 2-6 and SPR ranged from 0-5 in the subjects examined in the present study. Study done by Surekha et al\(^5\) showed more number of PPR in Kerala population as compared with Manipuri population. In addition, there was significant difference among different Sagittal Skeletal Malocclusion groups in terms of the overall number of PPR on either sides of the palate. Primary rugae were distributed more on the right side of the palate among the groups, especially in Skeletal Class II and Skeletal Class III. These results concur with those of Kallianpur et al\(^21\) who compared rugae patterns of Indian and Nepalese population. Rugae were higher in number on the right side of the palate in Indians as compared to Nepalese population but the results were not statistically significant. Dhoke and Osato\(^22\) and Kapali et al\(^13\), explained the phenomenon of regressive evolution that dominates the right side of the palate mainly pertaining to secondary rugae. Our results may thus vary due to the exclusion of secondary rugae and based on the population chosen.

According to Thomas 1983, palatal rugae do not undergo any changes except in length, throughout a person’s life probably due to underlying growth.\(^11\) Comparative studies performed amongst geographically diverse populations belonging to Indian regions such as Karnataka and Kerala\(^23\) and Manipur and Kerala\(^5\), have demonstrated important differences in rugae pattern. A study comparing two groups of Indian population (southern Indians and western Indians) predominantly showed the prevalence of wavy and curved rugae forms followed by straight rugae pattern.\(^24\) Studies conducted by Nayak et al\(^25\), Kotrashetti el al\(^26\), Kumar el al\(^27\), Surekha et al\(^8\), Shanmugam et al\(^7\), Bajracharya et al\(^28\) and Kapali et al\(^13\) showed the predominance of curved and wavy pattern of rugae in most populations. The Present study was aimed more towards assessing the characteristics of rugae in various skeletal malocclusions in Kerala population. Wavy pattern was seen most predominantly on both sides in Class I followed by Class II and Class III skeletal malocclusion. These are in accordance with the results published by Surekha et al\(^8\) and M Selvamani et al\(^29\) and SwethaS\(^30\). This was followed by curved, straight and unification pattern of rugae. Study by J K Savitha et al\(^23\) has seen similar results where curved rugae were more prominent in Kerala population as compared to Karnataka population. Horizontal rugae alignment and droplet-shaped incisive papilla was identified among all three skeletal dysplasia groups. The study by Ekrem Oral et al\(^10\) also reported droplet-shaped incisive papilla to be the most common type in Turkish population. M Selvamani et al\(^29\) concluded that females of Kerala origin had more number of primary rugae as compared to males. In our study we have not compared for sexual dimorphism among the various skeletal malocclusion. This leaves room for future scope of the study.

Further studies on a large number of subjects of varying Indian ethnicities are required in order to confirm the relationship between the pattern of palatal rugae and sagittal skeletal malocclusion.

**Conclusions**

- Primary rugae were significantly distributed more on the right side in comparison to the left side on the palate, especially in skeletal Class II and Class III.
- Wavy and curved pattern of the rugae were seen on both right and left side of all the skeletal malocclusion groups and skeletal Class III had more number of straight rugae.
- Direction of rugae alignment was horizontal and droplet-shaped incisive papilla was identified among different skeletal dysplasia groups.
- Palatal ruage may serve as valuable indicators for population identification and help in facial reconstructions through their association with varying skeletal malocclusions.

**Ethical Clearance:** Ethical clearance was obtained from the ‘Ethics Committee’ of the Institution prior to the start of the study.

**Source of Funding:** Self

**Conflict of Interest:** Nil

**References**


Analysis of Hospital Deaths at Tertiary Care Teaching Hospital

Jeeveswararao Bagadi
Srinivasulu Pothireddy
Sujan Kumar Mohanthy

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Abstract

Recording of Death and its causes constitute an important component of health information system. The study was conducted on 90 hospital deaths during the period of six months from July-December of 2017, at Great Eastern Medical School and Hospital. Males more than 60 years of age group were the major victims in our study. Improving primary health care facilities in rural areas may reduce the Mortality and Morbidity.

Key words:- Sudden Death, Mortality, Morbidity.

Introduction

Thanatology is a branch of science that deals with the study of death. A good death is not a single event; it is a series of events. Death analysis gives the circumstances and cause of death of patient and steps to be taken for prevention of same.

India is undergoing rapid transition as a consequence of economical and social reforms. Life expectancy at birth in India shows a continuous increasing trend from 23.63 years for male and 23.96 years for female in 1901 to 66.9 years for males and 70.0 years for females in 2011. The pattern of diseases in developing countries is different than developed ones. In India about 40% of deaths are from infectious, parasitic and respiratory diseases as compared with 8% in developed countries.

Death analysis determines the causes of major illness, quality of medical care provided to patients from analyzing the clinical records and hospital services. The pattern of mortality is a key indicator of the consequent health scenario.

Hospital based death records provide information regarding the causes of deaths, case fatality rates, age and sex distribution, which are of great importance in planning health care services. Mortality pattern is poorly documented in rural areas lacking retention of up-to-date medical records. In 19th and early 20th century communicable diseases dominated the health problems, in recent years non communicable diseases account for half of all deaths in developing countries. The main 4 killers of non communicable diseases are Cardio vascular diseases, Cancer, Diabetes and Chronic Lung Diseases.

Material and Method

A retrospective study was conducted at Great Eastern Medical School and hospital Srikakulam, a referral, tertiary care teaching hospital. All deaths that occurred during 6 months period from July to December of the year 2017 were considered for this study. Total 9,436 cases were admitted and 90 deaths were recorded. Age, Gender, Place of residence, Date of admission, Date of death, Survival period, Socio economic status, Family history, Past history, Chronic diseases, Proper basic patient care like CPR,ECG, were done or not, Consent taken or not, MCCD form properly filled or not and cause of death were recorded in this study. Approval of institution ethics committee was obtained prior to the study.

Table: 1 Social factors of hospital deaths

| Age >60 | 37.77% |
Table: 2 Age of patients in hospital deaths

<table>
<thead>
<tr>
<th>S.No</th>
<th>Age</th>
<th>No of Deaths</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0-10</td>
<td>12</td>
<td>13.33%</td>
</tr>
<tr>
<td>2</td>
<td>11-20</td>
<td>3</td>
<td>3.33%</td>
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<tr>
<td>3</td>
<td>21-30</td>
<td>7</td>
<td>7.77%</td>
</tr>
<tr>
<td>4</td>
<td>31-40</td>
<td>6</td>
<td>6.66%</td>
</tr>
<tr>
<td>5</td>
<td>41-50</td>
<td>13</td>
<td>14.44%</td>
</tr>
<tr>
<td>6</td>
<td>51-60</td>
<td>15</td>
<td>16.66%</td>
</tr>
<tr>
<td>7</td>
<td>&gt;60</td>
<td>34</td>
<td>37.77%</td>
</tr>
</tbody>
</table>

Table: 3 Time of Deaths

<table>
<thead>
<tr>
<th>S.No</th>
<th>Time</th>
<th>No of Deaths</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8AM -12PM</td>
<td>26</td>
<td>28.88</td>
</tr>
<tr>
<td>2</td>
<td>1PM – 8PM</td>
<td>36</td>
<td>40.00</td>
</tr>
<tr>
<td>3</td>
<td>9PM - 12AM</td>
<td>9</td>
<td>10.00</td>
</tr>
<tr>
<td>4</td>
<td>1AM – 7AM</td>
<td>19</td>
<td>21.11</td>
</tr>
</tbody>
</table>

Table: 4 Period of survival of patients in hospital deaths

<table>
<thead>
<tr>
<th>S.No</th>
<th>Time Period</th>
<th>No of Deaths</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt;24hrs</td>
<td>34</td>
<td>37.77</td>
</tr>
<tr>
<td>2</td>
<td>1-3 days</td>
<td>27</td>
<td>30.00</td>
</tr>
<tr>
<td>3</td>
<td>&gt;3-&lt;5 days</td>
<td>8</td>
<td>8.88</td>
</tr>
<tr>
<td>4</td>
<td>&gt;5-&lt;10 days</td>
<td>7</td>
<td>7.77</td>
</tr>
<tr>
<td>5</td>
<td>&gt;10-&lt;15 days</td>
<td>6</td>
<td>6.66</td>
</tr>
<tr>
<td>6</td>
<td>&gt;15-&lt;20 days</td>
<td>2</td>
<td>2.22</td>
</tr>
<tr>
<td>7</td>
<td>&gt;20 days</td>
<td>6</td>
<td>6.66</td>
</tr>
</tbody>
</table>

Table 5: Type of system involved in Hospital deaths

<table>
<thead>
<tr>
<th>S.No</th>
<th>System</th>
<th>No of Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Respiratory</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>Cardiovascular</td>
<td>21</td>
</tr>
<tr>
<td>3</td>
<td>Central nervous</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td>Gastro intestinal</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>Renal</td>
<td>7</td>
</tr>
<tr>
<td>6</td>
<td>Hematological</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>Endocrine</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>Multiple systems</td>
<td>9</td>
</tr>
<tr>
<td>9</td>
<td>Total</td>
<td>90</td>
</tr>
</tbody>
</table>

Table 6: List of major causes of death in hospital deaths

<table>
<thead>
<tr>
<th>S. no</th>
<th>Cause of death</th>
<th>No of deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Myocardial Infarction</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>Acute Respiratory Distress Syndrome</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>Pneumonia</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>Sepsis</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>Encephalopathy</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>Renal Failure</td>
<td>6</td>
</tr>
</tbody>
</table>
Observations & Discussion

Deaths were more common in the age group > 60 years (37.77%) followed by 41-60 years (31.11%). This study correlates with M M kauser et al, V M holamble. Lowest percentage of hospital deaths were recorded in the age group 11-20 years (3.33%). More deaths after 60 yrs can be explained by the pathological basis of disease, and decreased immunity in old age to infections.

More number of male deaths were recorded (61.11%) than female deaths (38.88%). This study correlates with M M kauser et al. This can be explained by more attention towards the health of male by family members. Deaths in Hindu religion (94.44%) can be explained by the more people belonging to Hindu religion in this area.

The highest numbers of Hospital deaths were in people belonging to rural area (88.88%) than urban area (11.11%). This may be because of location of the hospital is in rural area and serving more rural population. People from rural area are poor and referred from all types of medical centers like PHC, CHC, to medical college hospital in terminal stages of illness. People from urban area may have medical care from private sector. This study correlates with M M kauser et al, V M holamble.

More deaths were recorded in uneducated people (72.22%) than in educated people. This may be due to lack of knowledge on health in uneducated people in rural areas. In people with low socioeconomic status (84.44%) highest number of deaths was recorded followed by middle class people (11.11%). This may be due to poverty, poor people may be neglecting health and don't visit the hospital for screening and awareness programs. The highest number of deaths (n=62) were in married people than unmarried (n=28). More number of married people deaths can be explained by lower marriage age in rural areas.

People admitted with one of the chronic diseases (42.22%) were suffering from Hypertension, Diabetes, Thyroid, Carcinoma, which are prone to develop complications and early death. Past history of suffering from similar disease or other chronic disease was found in (27.77%) patients. In 5.55% of patients, family history of same disease was present. The patients who have not gone through any previous treatment or any screening procedures died more (62.22%), when compared with patients taking treatment (37.77%). The patients who are under treatment may take care of their health, there by prolonging the life. A positive history of alcohol and smoking was present in 22.22% of patients.

Highest numbers of deaths were recorded during day time (68.88%) than night time (31.11%). More number of deaths were recorded in people admitted in day time (72.21%) than night time (27.77%).

Deaths with in 24 hrs of hospital admission were more (n=34) in this study, implying the importance of screening procedures in rural villages of this district to prevent sudden deaths. Death is said to be sudden or unexpected when a person not known to have been suffering from any dangerous disease, injury or poisoning is found dead or dies within 24 hours after the onset of terminal illness. The incidence is approximately 10 percent of all deaths, which contrasts with our study (37.77%).

Diseases of Respiratory System killed more number of patients (n=26) followed by Cardio Vascular System (n=21), Central Nervous System (n=14), Gastro Intestinal System (n=8), Renal System (n=7), Hematology (n=3), Endocrine (n=2), and Multiple Systems (n=9). This study correlates with c.palaivel et al. contrasts with yogeshwar V. kalkonde et.al where stroke is the leading cause of death in rural people.

Myocardial Infarction and acute respiratory distress syndrome were the major immediate causes of death. This study correlates with c.palaivel et al. contrasts with yogeshwar V. kalkonde et.al where stroke is the leading cause of death in rural people.

In 75.55% of cases CPR was done and flat ECG was obtained in 81.11% cases and detailed consent was taken in 65.55% cases. MCCD forms were filled in 100% of cases.

Manner of death in 96.66 % of cases was natural and in 3.33% accidental.

Cadaveric organ donation was not done in this part of state even though 28 patients died below 40 yrs of age group in this study, due to lack of awareness.

In 18 cases, in MCCD forms immediate cause of death was written as cardio respiratory arrest. It shows the doctors are not well trained in MCCD form filling.

Conclusions

1. Deaths were more common in males aged more than 60 years.
2. Most of deaths occurred in rural, uneducated, low socio economic group people.

3. Respiratory diseases followed by Cardio vascular diseases accounted for highest number of deaths.

4. Acute respiratory distress syndrome and Myocardial infarction are the major immediate cause of deaths in respiratory and cardio vascular diseases respectively.

5. Diabetes and Hypertension were the common co morbid conditions observed in this study.

6. Significant number of deaths in young (n=15) people indicates importance of preventive measures like vaccines, screening methods etc.

7. Medical services were not available /used by most of the people before reaching this hospital.

8. More than one third of deaths (n=34) were sudden deaths i.e. <24 hrs of survival period after starting of the symptoms.

9. Most of deaths were natural deaths.

Suggestions and Recommendations

1. Higher mortality among males in rural areas will retard economic growth rates. So there should be strong health policy for preventive as well as curative health services.

2. Government should initiate better health awareness campaigns for healthy life styles, environment modifications, and safety measures in rural people.

3. Airway management, fluid resuscitation along with screening procedures for respiratory and cardiovascular diseases are the early contributors to prevent sudden deaths in hospitals.

4. Continuous evaluation in hospital records provides stimulation for improvement of clinical services, professional education, hospital administration and better patient care.

5. First three days after admission is better period which needs proper medical attention to avoid preventable deaths.

6. As hypertension and diabetes are the risk factor for Myocardial Infarction, screening and treatment of patients with hypertension and diabetes in rural areas reduces Myocardial Infarction mortality.

7. Delay in diagnosis and immediate treatment, and decision to transfer to higher centre may be the factors for deaths in rural area. Government should provide proper transport and should improve health facilities in rural area.

8. Late admissions, poor maintenance of health due to poor socioeconomic status may come under contributory negligence. Government should provide health workers in rural area to screen the people and avoid the late admissions.

9. Organ donation awareness programs should be conducted to improve organ donations in rural areas.

Acknowledgement: I am thankful to Dr Srinivasulu Pothireddy (Professor and Head), and Dr Sujan Kumar Mohanthy( Associate Professor), Department of Forensic Medicine and Toxicology, Great Eastern Medical College, for giving valuable suggestions during the study period.

Conflict of Interest: None

Source of Funding: Self

Ethical Clearance: Taken from Ethical committee, Great Eastern Medical College.

References


Study of Fingerprints in Relation to Dental Caries

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Abstract

Fingerprints of 40 individuals of age group 4-18 years were sampled from Agyaram Kherbari L.P. School, D. L. P School and J.N. M. E school (Dhubri District) to study the pattern of fingerprints and their relation with dental caries as they may be associated with the disease and serve as a predictor for early detection of it. Analysis was carried out in the affected individuals and it was found that the percentage of ulnar loop is highest (23.75%) in females and also in males (19.7%) and percentage of lateral loop is lowest (1.25% and 1.24%), whereas the control had more percentage of ulnar loop in both males and females. The study shows an association of dermatoglyphic pattern of fingerprints and prevalence of dental caries.

Key Words: Fingerprint patterns, Dental caries

Introduction

Fingerprints are impressions of skin ridges which are formed in the early embryonic life and remain permanent in each and every person including twins throughout life except changes during serious accidents. The physical attributes and its functions serve to identify a person and serve as a visible marker to predict different diseases. Fingerprints can be acquired from the fingers, toes, palm of hands and soles of feet and deployed in forensic as well as non forensic applications including identification and association of different diseases such as Diabetes mellitus, hypertension, congenital heart disease etc., from the authentication based on individual peculiarities and their unique individual specificity [¹²,⁷,⁸]. They are heritable and noted to play a significant role in human biology, research, medical field to study leukemia, breast cancer, in the field of dentistry, etc. Fingerprints can be a diagnostic tool in various pathologies of oral cavity and serve as a useful tool for preliminary investigations as it is heritable. Dactylography is a powerful diagnostic tool for fingerprint analysis to study psychological, medical and genetic conditions of individual. Since it is heritable it can be considered as a window for congenital abnormalities and an indicator of future diseases and one of the best available diagnostic tools to study genetic disorders. Due to their macrofeatures they can be classified variously and the important ones are arch, whorl, loops and composite types because the ridges are differentiated in their definitive form during third to fourth month of the fetal life which remains permanent throughout the whole life. The development of fingerprints is under genetic control and dermatoglyphics shows clear resemblance among related persons. Since dermatoglyphic features are genetically determined it can be now used as a tool to diagnose genetic disorders and to evaluate the relationship between different diseases whose relation may probably help us to identify an individual with high risk of developing a disease. As every part of the body is unique and synchronous with different parts, the distinctive fingerprint pattern can be a potential diagnostic tool for preliminary investigations for studying various diseases. It can be used as a screening tool for identifying patients who are likely to develop disease. Since different genes influence the enamel by its chemistry or morphology, fingerprints can be considered as an indicator of dental abnormalities [¹⁰, ¹³]. So the present study was carried out for studying the dermatoglyphic patterns of fingerprints of different age groups and to find the association of dermatoglyphic patterns with dental caries.

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Sonapur- 782402
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Material & Method

A total number of 40 school students in the age group of 4-18 years were selected from Agyaram Kherbari L.P. School, D. L. P School and J.N.M. E School (Dhubri District). A tabular chart was prepared before taking the fingerprint and clean finger imprints was taken for both hands on an A4 size paper by using a stamp pad for the Ink Method \[3\]. Sample fingerprints was then dried and studied with a magnifying lens with prior reference to identify the fingerprints. The fingerprint patterns were then classified according to the topological method \[5, 3\] for fingerprint identification.

Results

Among the 40 students, 20 students suffered from dental carries, out of which 10 were control girls and 8 were affected girls, and 7 were control males and 12 were affected males.

Fingerprints in control students

**Table 1.** establishes that in all control students, the highest per centage of fingerprint pattern in both hands was observed for radial loops (30.5%), followed by ulnar loop (28.5%), plain whorl (8%), double cored, plain whorl (7.5%), double loop (5.5%), spiral whorl (5%), tented arch (4.5%), plain arch (3.5%), central pocket loop whorl (2.5%), elliptical whorl (1.5%), lateral pocket loop (1%) and accidental whorl (1%). The left hand shows a higher per centage (30.5 %) radial loop count in control and the ulnar loop count is 28.5% for right hand.

Fingerprints in affected students

The highest per centage of fingerprint pattern as shown in **Table 1.** for both hands was observed for ulnar loop (21%), followed by radial loop (20%), spiral whorl (18.5%), double cored whorl (9%), elliptical whorl (8%), plain arch (7.5%), plain whorl (7%), double loop (4.5%), tented arch (2%), central pocket loop whorl (2%) and lateral pocket loop (0.5%) in affected students. The left hand shows a less per centage of radial loop count in affected individuals results with no occurrence of radial loop. The right hand shows highest ulnar loop count (28.5%) and in affected the count is 21 %.

The fingerprint patterns of affected female and male students are graphically represented in **Fig.1** and **Fig. 2.** **Table 1.** also shows the fingerprint per centage of control female and affected female.

Fingerprints in control female students

The fingerprints of control female in **Table 2.** shows radial and ulnar loop (28%), plain whorl (11%), double cored whorl and tented arch (7%), spiral whorl (5.4%), double loop (3.9%), central pocket loop whorl and plain arch (3%), accidental whorl (1.54%) and lateral pocket loop (1.54%), elliptical whorl (0.8%).

Fingerprints in affected female students

The fingerprints of affected female shows ulnar loop (23.75%), radial loop (22.5%), spiral whorl (17.5%), plain arch (10%), double cored whorl (8.75%), plain whorl (6.5%), tented arch (3.75%), double loop (2.5%), central pocket loop whorl (1.25%), lateral pocket loop (1.25%). However accidental whorl is not observed in affected female (**Table 2**).

Fingerprints in control male students

As observed in **Table 2** in control male the highest per centage of fingerprint is found in radial loop (35.72%), followed by ulnar loop (30%), double cored whorl (8.57%), double loop (8.6%), spiral whorl (4.2%) and plain arch (4.2%), plain whorl (2.85%) and elliptical whorl (2.85 %), central pocket loop whorl (1.43%). However, accidental whorl and tented arch is not observed in control male.

Fingerprints in affected male students

In affected male ulnar loop is found to be highest (19.17%), followed by radial loop (18.33%), spiral whorl (19%), elliptical whorl (13%), double cored whorl (9%), plain whorl (6.67%), double loop (5.9%), plain arch (5.9%), central pocket loop whorl (2.5%), tented arch (0.84%). Accidental whorl is not observed (**Table 2**).
Table 1 - Comparative study of fingerprint patterns of control and affected students

| Sl. No | Fingerprint patterns | Left Hand |  | Right Hand |  | Both Hands |  |
|--------|----------------------|-----------|-----------------|-----------|-----------------|-----------|
|        |                      | No of Control | % | No of Affected | % | No of Control | % | No of Affected | % | No of Control | % | No of Affected | % |
| 1      | Radial loop          | 61         | 30.5 | 40 | 20 | 0 | 0 | 0 | 0 | 61 | 30.5 | 40 | 20 |
| 2      | Ulnar loop           | 0          | 0 | 0 | 0 | 57 | 28.5 | 42 | 21 | 57 | 28.5 | 42 | 21 |
| 3      | Lateral pocket loop  | 1          | 0.5 | 0 | 0 | 1 | 0.5 | 1 | 0.5 | 2 | 1 | 1 | 0.5 |
| 4      | Double loop          | 4          | 2 | 6 | 3 | 7 | 3.5 | 3 | 1.5 | 11 | 5.5 | 9 | 4.5 |
| 5      | Plain whorl          | 7          | 3.5 | 5 | 2.5 | 9 | 4.5 | 9 | 4.5 | 16 | 8 | 14 | 7 |
| 6      | Spiral whorl         | 7          | 3.5 | 20 | 10 | 3 | 1.5 | 17 | 8.5 | 10 | 5 | 37 | 18.5 |
| 7      | Double cored whorl   | 7          | 3.5 | 11 | 5.5 | 8 | 4 | 7 | 3.5 | 15 | 7.5 | 18 | 9 |
| 8      | Elliptical whorl     | 0          | 0 | 5 | 2.5 | 3 | 1.5 | 11 | 5.5 | 3 | 1.5 | 16 | 8 |
| 9      | Central pocket loop  | 2          | 1 | 1 | 0.5 | 3 | 1.5 | 3 | 1.5 | 5 | 2.5 | 4 | 2 |
| 10     | Accidental whorl     | 1          | 0.5 | 0 | 0 | 1 | 0.5 | 0 | 0 | 2 | 1 | 0 | 0 |
| 11     | Plain arch           | 4          | 2 | 9 | 4.5 | 3 | 1.5 | 6 | 3 | 7 | 3.5 | 15 | 7.5 |
| 12     | Tented arch          | 6          | 3 | 3 | 1.5 | 3 | 1.5 | 1 | 0.5 | 9 | 4.5 | 4 | 2 |

Table 2. Comparative study of fingerprint patterns of control and affected female and male students

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Fingerprint patterns</th>
<th>Difference in percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Normal individuals (%)</td>
</tr>
<tr>
<td>1</td>
<td>Radial loop</td>
<td>30.5</td>
</tr>
<tr>
<td>2</td>
<td>Ulnar loop</td>
<td>28.5</td>
</tr>
<tr>
<td>3</td>
<td>Lateral pocket loop</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>Double loop</td>
<td>5.5</td>
</tr>
<tr>
<td>5</td>
<td>Plain whorl</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>Spiral whorl</td>
<td>5</td>
</tr>
</tbody>
</table>
Table 2. Comparative study of fingerprint patterns of control and affected female and male students

<table>
<thead>
<tr>
<th>Pattern Type</th>
<th>Control</th>
<th>Affected</th>
<th>Control</th>
<th>Affected</th>
<th>Control</th>
<th>Affected</th>
<th>Control</th>
<th>Affected</th>
<th>Control</th>
<th>Affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Double cored whorl</td>
<td>7.5</td>
<td>9</td>
<td>7</td>
<td>8.75</td>
<td>8.57</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elliptical whorl</td>
<td>1.5</td>
<td>8</td>
<td>0.8</td>
<td>0</td>
<td>2.85</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Central pocket loop whorl</td>
<td>2.5</td>
<td>2</td>
<td>3</td>
<td>1.25</td>
<td>1.43</td>
<td>2.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accidental whorl</td>
<td>1</td>
<td>0</td>
<td>1.54</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plain arch</td>
<td>3.5</td>
<td>7.5</td>
<td>3</td>
<td>10</td>
<td>4.2</td>
<td>5.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tented arch</td>
<td>4.5</td>
<td>2</td>
<td>7</td>
<td>3.75</td>
<td>0</td>
<td>0.84</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Study of fingerprints of control and affected females.

Fig. 2. Study of fingerprints of control and affected males.
Discussion

Our study reveals, the fingerprint patterns varied among the students affected with dental caries. The loop pattern of fingerprint was found to be the highest and more prevalent in students having dental caries \[9, 11\] compared to fingerprint patterns in normal unaffected students. This study also clarifies that among the loops, ulnar loop (21\%) was the most prevalent.

As per our findings per centage of loops are followed by whorls in affected individuals and our study is in contrast to the other observations \[6, 4\] wherein, prevalence of dental carries was highest among students with whorl pattern compared to other fingerprints.

In this study the left hand of individuals showed a higher per centage (30.5\%) radial loop count in control compared to affected individuals (20\%) with no occurrence of radial loop in the left hand. The results are in accordance with workers \[11\] who also found radial loops among the control and the results are also at par with some similar works \[2\] as more number of radial loops is considered as a negative indicator for dental caries. The right hand however, shows highest ulnar loop count (28.5\%) in control individuals and lowest in affected (21 \%) individuals, which may indicate that the radial loops are more abundant in left hand compared to right hand. Our study also reveals higher percentage of ulnar loops (21 \%) compared to radial (20\%) when both hands are compared and ulnar loop fingerprint pattern can be a predictor for dental carries which however contradicts certain results \[2\] where it was found that whorl pattern shows positivity for dental caries prediction.

Comparative study between normal female and affected female also shows more number of ulnar loops (23.75\%) in students affected with dental caries compared to normal females who had equal per centages of radial loop (28\%) and ulnar loop (28\%). The results of this study also shows similarity with the affected males with the highest per centage of ulnar loop (19.17\%) followed by radial loop (18.33\%) compared to control males with radial loop (35.72\%).Thus ulnar loops may be an indicator to assess the susceptibility of an individual to dental carries.

Comparative studies also show a difference in the per centage of whorl fingerprint pattern. Spiral whorl pattern has a difference in which the per centage of affected is more (37\%) and in case of normal it is very less (5\%). Similarly, elliptical whorl for affected is 8\% and for normal it is 3\%, accidental whorl is present in unaffected individuals but is absent in affected individuals. Our result corroborates the results \[2\] which established the prevalence of dental caries with whorl pattern.

Conclusions

It is hereby concluded from our research that

1. Frequency distribution of fingerprint patterns among normal students differs from affected students.

2. Frequency of ulnar loop pattern is more prevalent followed by radial loop pattern in individuals with dental carries, with no occurrence of accidental whorls. This may serve as a predictor between fingerprint patterns and dental caries. The frequency of ulnar loop is more in right hand compared to left hand followed by the radial loop pattern. More radial loops are a negative indicator for dental caries. Spiral whorls can also be a predictor for dental carries as it is followed by the ulnar loop in affected individuals.

3. Out of the 40 individuals, 10 males and 10 females were affected which may indicate equal occurrence in both and this might be genetical or oral hygiene in the age groups of 6-14 years students which however, require further study.

4. Thus dermatoglyphics gives a hope in the prediction of dental caries much before its initiation giving a sufficient time to implement preventive measures. So further studies is required to be carried out in the field of medicine with their related genetical studies and more comparative studies with large samples

Conflict of Interest: None Declared.

Source of Support: Nil

Ethical Clearance- Taken from Biosafety Committee

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Trends & Pattern in Unnatural Female Death Cases Due to Burn: A One Year Retrospective Study

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Abstract

Burns are the injuries that are produced by application of dry heat such as flame, radiant heat or some heated soiled substance like metal or glass from the surface of the body resulting in tissue destruction. According to WHO estimates about 2,65,000 deaths occur each year from fires alone globally, with more deaths from scalds, electrical burns, and other forms of burns for which data are not available. The majority of these deaths occur in low- and middle-income countries, with almost half occur in the WHO South-East Asia Region. In India around 7 million people suffer from burn injuries each year with 1.4 lakh deaths and 2.4 lakh people suffer with disability. Burn death rates have been decreasing in high income countries. Burn injury are higher in females than males. The higher risk for females is associated with open fire cooking, or unsafe cook stoves, loose clothing. Self-directed or interpersonal violence is also a factor for burn injuries. The present study is retrospective study which was carried out in the Department of Forensic Medicine and Toxicology at B. J. Medical College and Civil Hospital, Ahmedabad during the period from January 2018 to December 2018 to determine trends & pattern in unnatural female death due to burns cases and their practical approach to shrinkage the incidence of female burns cases as much as possible. In study we come across some particularized factor which exaggerated the recent condition in the society. The various facets are investigated and conferred in specifics.

Keyword: Unnatural female death, thermal injury, Septicemia

Introduction

Burns are a critical public health problem, causing deaths, disability and disfigurement. Globally, there are about 300,000 deaths due to burns every year. Of these, 95% take place in developing countries with Southeast Asia recording nearly 57% of deaths due to burns.¹ World Health Organization (WHO). A who plan for burn prevention and Care. 2008; WHO: Geneva.² Extrapolation of data from major hospitals indicates an estimation of 7 million burn incidents in India each year, making burn injuries the second largest group of injuries after road accidents. In 1998, India was the only country in the world where fire was among the 15 leading causes of death, according to WHO.³ However, the Government of India has not put in place a national injury surveillance system, hence the exact incidence of burn-related morbidity and mortality is not known. In 2010, the Government of India announced the National Programme for Prevention of Burn Injuries (NPPBI) which aims at prevention, burns injury management and establishment of a central burn registry, but its impact is not yet noticeable.⁴ A study of women’s health priorities based on the 2004 mortality estimates and disease burden of the WHO Global Burden of Diseases Study found that burns in young women were common in Southeast Asia, with it being the third cause of death for women aged 15–44, followed by self-inflicted injuries.⁵ Burn-related injuries and deaths amongst women in India are likely to be caused by: kitchen accidents related to use of kerosene and flammability of garments; self-immolation or suicides; and homicides related to domestic violence. Evidence suggests that domestic violence (physical, sexual and emotional), which is widespread in India, could have an important role in these burn injuries.⁶
The NCRB records show 122,877 cases of violence by husband or relatives, 8,455 dowry deaths and 2,233 suicides among women due to dowry harassment in 2014. The NCRB data is based on cases registered with the police and these numbers are therefore grossly underreported and may not provide accurate data on burn injuries. A burn injuries death is very excruciating but what compels or in what surroundings women commits suicide or homicide or those accidentally burned but most heinous is burning of newly married women i.e. homicidal burning. In this respect it is very difficult to find out the manner (Suicidal, Accidental and Homicidal) of burn injuries that in what circumstances the burn injuries took place, it can only be possible by meticulous investigation of scene of crime and interrogation of person concerned.

Aims & Objective

To find out burn affect in respect to sex, religion, season, time of incidence, marital status, total body surface area distribution (TBSA), Duration of survival, alleged history, manner & cause of death wise distribution and Its medico-legal consequence.

Materials & Method

This study was carried out in the Department of Forensic Medicine and Toxicology at B. J. Medical College and Civil Hospital, Ahmedabad during the period from January 2018 to December 2018. The Proforma of the study was prepared and numerous information and conclusions were collected & analysed from Post-Mortem report records & hospital data records. Data has been analysed in respect to sex, religion, season, time of incidence, marital status, total body surface area distribution (TBSA), Duration of survival, alleged history, manner & cause of death wise distribution. The details were accumulated, tabulated, discussed & concluded.

Observations & Result

Total 3930 Autopsy were conducted in B.J Medical college, Ahmedabad during during the period from January 2018 to December 2018 among them 816 (20.76%) cases are unnatural female death cases. Unnatural Female death cases due to burn(Thermal injury)(64.25%) are near to double than the unnatural death cases due to burn male death (35.75%) cases. It shows the definite crucial factor in gender-biased in the society. Religion wise distribution of burn female cases shows more incident in Hindu Female(48.81%), and Muslim Female(39.89%) than others. Hence highest in the Hindu religion among all. Unnatural burn(Thermal) injury cases are more predominant in winter(Nov-Feb) (46%) & Summer(March-June)(40%) than in rainy season(July-Oct)(14%) Incidence of unnatural female death due to burn cases are more prone in Morning(6am-12 pm)(37%) & Evening(6pm-12am) (39%), as they are more active in cooking related activities where incidence of accidental burns are more prominent. Triple times of incidence of unnatural female death due to burn in female in married female (64%) than unmarried(23%) is noted as dowry and other social factors are responsible in married female.

Averagely 30-40% of Total body surface area (TBSA)(21%) & 40-50% of TBSA(20%) involved in unnatural female death cases and lesser in 10-20% (1%) & >80%(5%).

<table>
<thead>
<tr>
<th>Table No 1: Total Body Surface Area Involved: percentagewise Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total body surface area involved</td>
</tr>
<tr>
<td>10-20%</td>
</tr>
<tr>
<td>20-30%</td>
</tr>
<tr>
<td>30-40%</td>
</tr>
<tr>
<td>40-50%</td>
</tr>
<tr>
<td>50-60%</td>
</tr>
<tr>
<td>60-70%</td>
</tr>
<tr>
<td>70-80%</td>
</tr>
<tr>
<td>&gt;80%</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

Duration of survival after incidence of burn(Thermal) Injury is least in more than one month (5%) & more in 12 to 24 hr(19%) & 24 to 48 hr (17%) (Table no.2)
Table No 2: Duration of Survival after Incidence:

<table>
<thead>
<tr>
<th>Duration of survival after incidence</th>
<th>Total burn cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brought dead</td>
<td>38</td>
<td>10%</td>
</tr>
<tr>
<td>0 to 6 h</td>
<td>22</td>
<td>6%</td>
</tr>
<tr>
<td>6 to 12 h</td>
<td>50</td>
<td>13%</td>
</tr>
<tr>
<td>12 to 24 h</td>
<td>72</td>
<td>19%</td>
</tr>
<tr>
<td>24 to 48 h</td>
<td>65</td>
<td>17%</td>
</tr>
<tr>
<td>48 to 72 h</td>
<td>41</td>
<td>11%</td>
</tr>
<tr>
<td>3 days to 7 days</td>
<td>49</td>
<td>13%</td>
</tr>
<tr>
<td>7 days to 1 month</td>
<td>22</td>
<td>6%</td>
</tr>
<tr>
<td>More than 1 month</td>
<td>19</td>
<td>5%</td>
</tr>
<tr>
<td>Total</td>
<td>381</td>
<td>100%</td>
</tr>
</tbody>
</table>

Previous history of suicidal attempts (27%) are more leads to unnatural burn injury death cases, also psychiatric illness (10%), chronic illness (13%) are more to deal with suicidal female burn death cases. (Table No.3)

Table No 3: Alleged History as Discussed with Relative in Burn Cases

<table>
<thead>
<tr>
<th>History</th>
<th>Female</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psychiatric illness</td>
<td>40</td>
<td>10%</td>
</tr>
<tr>
<td>Chronic illness</td>
<td>57</td>
<td>13%</td>
</tr>
<tr>
<td>Previous suicidal attempts</td>
<td>52</td>
<td>27%</td>
</tr>
<tr>
<td>Addiction</td>
<td>60</td>
<td>15%</td>
</tr>
<tr>
<td>NIL</td>
<td>172</td>
<td>45%</td>
</tr>
<tr>
<td>Total</td>
<td>381</td>
<td>100%</td>
</tr>
</tbody>
</table>

Manner of death due to burn are more common in accidental cases(41%), & suicidal cases(24%) as per history taken by relatives & Inquest papers. (Table no.4)

TABLE NO 4: Manner of Death as per inquest & history

<table>
<thead>
<tr>
<th>Manner of Death as per inquest</th>
<th>Female Burn cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accidental</td>
<td>156</td>
<td>41%</td>
</tr>
<tr>
<td>Homicidal</td>
<td>72</td>
<td>19%</td>
</tr>
<tr>
<td>Suicidal</td>
<td>91</td>
<td>24%</td>
</tr>
<tr>
<td>other not differentiated</td>
<td>62</td>
<td>16%</td>
</tr>
<tr>
<td>Total</td>
<td>381</td>
<td>100%</td>
</tr>
</tbody>
</table>

TABLE NO 5: Cause of death in female burn cases distribution

<table>
<thead>
<tr>
<th>Cause of death</th>
<th>Due to</th>
<th>No. of cases</th>
<th>Percentage%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burns</td>
<td>septicemia+pneumonia +acute renal failure</td>
<td>106</td>
<td>28%</td>
</tr>
<tr>
<td></td>
<td>Exhaustion</td>
<td>30</td>
<td>8%</td>
</tr>
<tr>
<td></td>
<td>Neurogenic shock</td>
<td>98</td>
<td>25%</td>
</tr>
<tr>
<td></td>
<td>Electric shock</td>
<td>27</td>
<td>8%</td>
</tr>
<tr>
<td></td>
<td>asphyxia</td>
<td>41</td>
<td>11%</td>
</tr>
<tr>
<td></td>
<td>hypovolumic shock</td>
<td>53</td>
<td>14%</td>
</tr>
<tr>
<td></td>
<td>Multi-organ failure</td>
<td>22</td>
<td>6%</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>381</td>
<td>100%</td>
</tr>
</tbody>
</table>

Discussion

The present retro-spective study which was carried out in the Department of Forensic Medicine and Toxicology at B. J. Medical College and Civil Hospital, Ahmedabad during the period from January 2018 to December 2018 to determine trends & pattern in unnatural female death due to burns cases and their practical approach to shrinkage the incidence of female burns cases as much as possible. In study we come across some particularized factor which exaggerated the recent condition in the society.

In a total of 381 cases of burns, females formed the majority with 64.25% which is in harmony with the
studies of Chawla et al.17, Mazumdar et al.8, Aggarwal9 and Chandra, Doshi10, Ganguly11, Sinha et al.12, Haralkar and Rayate13, Naralwar and Meshram14. The reason may be accredited to the fact that women in India are accountable for the domestic duties including cooking where due to cultural and religious motives they wear dangerously long and loose fitted clothing. This together with lack of safe installation of cooking appliances that involves chullah, kerosene stove burst and gas leakage, making them the more vulnerable gender.

Majority of the burn victims were Hindus (48.81%) of the total burn Female victims) which is similar to the findings of D. Nath15 P.Singh16. The reason being Hinduism is the largest religion practised in India where the previous dowry system and “sati pratha” still existed in rural area.

Seasonal variations in our study showed that burn deaths occurred mostly in winter(46%) followed by summer(40%).17 This might be due to the fact that, in winter, there is more need for hot water for bathing. The traditional kerosene stove; which is extensively used in the slum areas city for cooking and providing the necessary boiling water for bathing; lacks any safety measures more in Morning (37%) & evening(39%).

Thus, it is condemned to be responsible for much of the flame and scald burns in our country. This fact has been previously highlighted by the work of reporters from low income countries.18

The domestic violence can be further reinforced by the marital status of the genders in this study in which, the majority of the female death due to burn victims were married (64%) than unmarried(23%).19 Average 30-40% of TBSA(21%) & 40-50% of TBSA(20%) involved in unnatural female death cases.

Though the majority of the incidents are accidental in nature, suicidal and homicidal cases were also observed. As noted with other studies, accidental burning was the commonest manner of deaths due to burning followed by suicidal and homicidal burning.20-23 More incident noted in Previous suicidal attempt(27%) than Psychiatric illness(10%),chronic illness(13%),addiction(15%) and more in accidental(41%) than suicidal(24%)

Shock is found to be the most common cause of death in most of the victims, which is similar to the findings of Chawla R, Chanana A, Rai H, Aggarwal A.D et al7, Singh P, Sharma16 and D. Nath.15 Shock (neurogenic[25%], hypovolemic[14%]) is more common in 1-2 day period after burn injury. Any kind of injury including burn injury was the common source of infection, which resulted in septicaemia,pneumonia,renal failure and septicaemic death. Lack of care on the part of the doctor was the main reasons for the septicaemic deaths in the burn victims.

### Conclusion

Female(64.25%) are more prone to death due to Burn (Thermal) injury than male(35.75%) in this retrospective study almost near double. Hindu Female(48.81%) & Muslim female (39.89%) are more indulge to death due to Burn(Thermal) injury than others. There is increased incidence of death due to burn injury in summer(March-june) (40%) & winter (Nov-feb) (46%) than in rainy season. There is increased incidence of death due to burn in Morning(37%) & evening(39%) as Female are more active in cooking during this time period. Married woman(64%) are more prone to thermal injury than unmarried & widow. Averagely 30-40% of TBSA(21%) & 40-50% of TBSA(20%) involved in unnatural female death cases. Duration of survival after incidence is least in more than one month. Alleged history of previously attempted suicidal attempt (27%) are more prone to burn(Thermal) injury for unnatural female death. Accidental burn (41%) cases are more predisposed than others. Death due to burn are more (28%) in septicaemia,pneumonia ,acute renal injury & Neurogenic shock (25%).

### Ethical Clearance:

Taken From Ethical committee, Civil Hospital, Ahmedabad

### Conflict of Interest:

None.

### Source of Funding:

None.

### Contribution of authors:

We declare that this work was done by the author(s) in this article and all liabilities pertaining to claim relating to the content of this article will be borne by the authors. The study was conceived and designed by Dr Manjit Nayak; data collection and analysis by Dr Manjit Nayak, Dr.Saumil Merchant, Dr.Kalpesh shah

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Effectiveness of Structured Exercise Programme Versus Elastic Band Exercice on Individuals with Rounded Shoulder

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Abstract

Background: Rounded shoulder is a common postural abnormality in young adults due to abnormal posture adaptation while working. It may lead to decrease in thoracic mobility and increase in intradiscal pressure. In severe cases breathing problem may occur due to changes in the anterio-posterior diameter. Few studies have shown the effect of elastic band exercise on rounded shoulder to improve the postural alignment. Shoulder stabilization exercises and pectoralis minor stretching is also effective in rounded shoulder. In these study structured exercise were use to correct the rounded shoulder posture and to prevent the further complication.

Objective: To find out the effectiveness of structured exercise programme on subjects with rounded shoulder.

Material and method: In this pre-post intervention study 32 individuals with rounded shoulders were included. They were randomly divided into 2 groups with 16 individuals in each group. Group A were treated with structured exercises and Group B were treated with elastic band exercises for 4 weeks. After pre-post assessment, data was analysed with the help of appropriate statistical methods.

Results: According to the statistical analysis there is significant decrease in measurement of rounded shoulder in both Group A and Group B with p value <0.0010 and <0.0001 respectively.

Conclusion: Structured exercise and elastic band exercises both are equally effective in improving the rounded shoulders.

Keywords: Rounded shoulders, vernier calliper, structured exercises and elastic band exercises.

Introduction

Rounded shoulder posture is the commonest abnormal structural anomalies of the shoulder complex. Incidence of rounded shoulder posture is increasing in young adults due to repetitive work and bad posture while working.[1] It is a posture in which scapulae are elevated and the acromion processes are protruded forward to compromise the centre of gravity. It involves anterior tilt of the cervical vertebra and the posterior tilt upper thoracic vertebra which leads to shoulder protraction, turn downwards and anterior tilting.[1,4]

Any changes in the skeletal alignment can indicate changes in antagonist and agonist muscles, imbalance in stretching and shortening of muscles. Inappropriate posture may aggravate the pain and soft tissue damage. Rounded shoulder increases the thoracic kyphosis which abdominal muscles may shorten and the anterior part of vertebral bodies is compressed it leads to increase in the intradiscal pressure.[1,11]

Stretching of trapezius muscle is also seen in this individual due to scapular protraction. In individuals with this condition there is shortening of pectoralis
minor due to adaptation and through the restriction of the scapular motion.[1,12,13,14]

In this study to assess the rounded shoulder vernier calliper was used. Patient position is supine and examine the distance between plinth and the acromion process with vernier caliper. If the distance is > 2.5 cm then the individuals are included in this study.[2,19]

Posture correction programs can correct the postural alignment and help in decreasing the pain. In this study elastic band exercise and structured exercise programme was used to correct the rounded shoulders. Velocity and intensity of elastic band can be varied. It is used to improve the muscular strength, flexibility and balance control. Elastic band exercise programme consists of the following exercises – lateral pull down, shoulder external rotation, shoulder horizontal abduction exercise, a seated bend row, shoulder abduction exercise, shoulder flexion exercise, shoulder extension exercise. 1) For lateral pull down subjects held both the ends of elastic band and stretch the band in sideway direction and pull it down to their chest, abdominal muscles should be contracted while performing this exercise. 2) To perform shoulder external rotation subjects should be in standing position and their elbow in 90 degree, palm towards the ceiling. Elastic band should be stretch in outward direction while performing this, elbow should be in stable position. 3) Shoulder horizontal abduction – subjects extend their arm in front of their body at 90° and place the shoulder width apart. Their palm should face down and held the elastic band and slowly stretch the elastic band horizontally keep elbow straight while performing it. 4) In seated bend row, the subjects place the elastic band in such a way that their leg should be in middle of the band and stretch the band towards body. 5) Shoulder abduction exercise – subjects should keep one feet on the elastic band and another end of elastic band should be hold on one hand after that stretch it out in outward direction or away from the body. 6) For the shoulder flexion exercise kept one end of elastic band under feet and another end hold in hand, perform shoulder flexion with elbow straight. 7) Shoulder extension exercise, the examiner hold one end of elastic band and other end hold by the patients and ask them to extend the arm backward with elbow straight as much as possible.[3]

In structured exercise programme exercises consists of- chin tucks, reverse plank, cat and camel, Bhujangasana ( cobra pose), wall push ups, shoulder blades squeeze. Chin tucks is exercise to improve the neck strength, flexibility and neck function. To perform it subjects should sit upright, place a finger on chin then pull the chin until the stretch is felt. Hold it for 10 seconds and repeat for 10 times, per session. Reverse plank is done in supine position subject should be in supine position place a palm on floor slightly behind hips. Press the palm and lift the hips towards ceiling, squeeze the core muscle and hold it for 5-10 seconds. To perform cat and camel subjects should be in quadrupod position, slowly alternate between arching and rounding your back so that spine extend and flex alternately. In cobra position subjects should patients should be in prone lying gently lift the forehead, elbow should be straight while performing it and hold the position for 10 seconds and it will be done for 10 times. Wall push ups- patient should stand in front of wall keep one arm distance between wall and patients then palm should be rest on wall after that gently try to push the wall and come forward towards wall. Shoulder blade squeeze- in standing position patient should keep arm at sides then squeeze the shoulder blade together.[5,6,7,8]

Rounded shoulder may cause complications like increase in intradiscal pressure, decrease in thoracic mobility and in severe cases there may be breathing problems. Rounded shoulder should be corrected with the help of stretching and strengthening exercise of the scapular muscles to prevent the further complications.

**Methodology**

An experimental study was carried out using pre and post study design. The place of the study was Krishna institute of medical sciences, Physiotherapy department, Karad. There were 32 participants in the study with rounded shoulders. The samples were chosen using the simple random sampling method. Participants was included as per inclusion and exclusion criteria.

**Procedure**

Subjects were selected for the study according to the selection criteria. Demographic data and consent form was taken from them. Included participants was divided into two groups by random sampling method. Pre and post assessment was taken before and after 4 weeks of the treatment respectively with the help of outcome measures. Group A received structured exercise programme, 15 repetitions of 3sets, 3 times per week for 4weeks. Structured exercises consist of chin tucks, reverse plank, cat and camel, Bhujangasana ( cobra pose), wall push ups, shoulder blades squeeze,
shoulder shrug.

Group B received elastic band exercise consists of lateral pull down, shoulder external rotation, shoulder horizontal abduction exercise, a seated bend row, shoulder abduction exercise, shoulder flexion exercise, shoulder extension exercise. Each exercise are for 15 repetitions of 3 sets per session, 3 session per week. The effect of each treatment were assess after 4 weeks using outcome measures.

The effect of exercises of the group was noted with the help of outcome measures. The experimental results was statistically analysed. The significant difference between the two groups was investigated with the unpaired t test and within the group with paired t test.

### Findings

Pre and post data was analysed according to the result within group A of both right and left side and it considered extremely significant with p value <0.0010. The mean ± SD of pre assessment of left and right side is 6.36 ± 1.17. Mean ± SD of post assessment of left and right side is 5.80 ± 1.06 (fig. no. 1).

The pre and post data was also analysed according to the result within group B of both right and left side and it considered extremely significant with p value <0.0001. The mean ± SD of pre assessment of left and right side is 5.90 ± 0.95. Mean ± SD of post assessment of left and right side is 5.73 ± 0.91 (fig. no. 2).

The mean ± SD of post intervention of group A is 5.80 ± 1.06 and of group B is 5.73 ± 0.91 (fig. no. 3).

### Discussion

This study aimed to investigate the effectiveness of structured exercise programme versus elastic band exercise on individuals with rounded shoulder with an objective of finding the benefits of each exercise and comparing its effects to fulfil the aim of the study.

The individuals with rounded shoulders are assessed with the help of vernier caliper. There were 32 participants which were selected according to the inclusion and exclusion criteria and they were randomly divided into 2 groups (group A and group B) with 16 in each group. Group A was asked to perform structured exercises and group B was asked to perform elastic band exercises. Pre and post assessment were taken prior to treatment with the help of vernier calliper. In structured exercise programme exercises included are chin tucks, reverse plank, cat and camel, Bhujangasana (cobra pose), wall push ups, shoulder blades squeeze. Elastic band exercises are lateral pull down, shoulder external rotation, shoulder horizontal abduction exercise, a seated bend row, shoulder abduction exercise, shoulder flexion exercise, shoulder extension exercise. The study was carried out for 4 weeks 5 times in a week. After the pre
and post assessment the data was statistically analysed.

The result indicated that both the exercise groups appeared to be equally effective in improving the rounded shoulder posture. Various study showed that stabilization exercises and stretching exercises are helpful in improving rounded shoulder posture\(^1\). According to the study by author Kim TW, An DI, Lee HY, Jeong HY, Kim DH, Sung YH\(^2\) which was on effects of elastic band exercise on subjects with rounded shoulder posture and forward head posture suggest that elastic band exercises are effective in improving the rounded shoulder posture.

**Conclusion**

On the basis of the result it is concluded that the structured exercises and elastic band exercises are equally effective in improving the posture in individuals with rounded shoulders. The impact of these exercises on the rounded shoulders needs to be taken into consideration.

**Acknowledgement:** I sincerely thank the management of KIMSDU for allowing me to conduct this study by providing me the necessary requirements. I thank dean Dr Varadharajulu sir for his support and guidance. My sincere thanks to guide Dr Khushboo Chotai for helping me in my research. I take this opportunity to thank all those who have been directly or indirectly involved for smooth conduction of this study.

**Conflict of Interest:** There were no conflicts of interest in my study.

**Source of Funding:** Funding is given by Krishna Institute of Medical Sciences, “Deemed to be” University, Karad.

**Ethical Clearance:** The Institutional Ethics committee has hereby given permission to initiate the research project titled, “Effectiveness of structured exercise programme versus elastic band exercise on individuals with rounded shoulder”.

**References**


Effectiveness of Cognitive Therapy in Post-Menopausal Women

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Abstract

Objective: To find the effectiveness of cognitive therapy in post-menopausal women.

Method: The study group consisted of 50 post-menopausal women, aged between 45-65 years, screening of 100 women was done and 50 women having cognitive changes and fulfilling the inclusion and exclusion criteria were selected. They were given exercises for 4 weeks, 4 times per week and pre-post result were calculated on the basis of attention, memory, anxiety and depression. The scores were measured by using mini mental scale, mood scale and Hamilton depression rating scale.

Conclusion: The results demonstrated that there is statistically extremely significant difference in cognitive changes in post menopausal women. (p >0.0001)

Key Words: post-menopausal, women, cognitive changes.

Introduction

Menopause is defined as time of cessation of ovarian function resulting in permanent amenorrhea. The climacteric is the phase of weaning ovarian activity, and may begin 2-3 years before menopause and 3-4 years after menopause, it also involves physical, sexual and psychological adjustments. Menopause in India generally occur at the age of 45-50 years (mean age 47 years). During this phase ovarian activity declines, no corpus luteum is formed and no progesterone is formed. Later graafian follicles fail to develop and estrogenic activity is reduced¹.

Due to these changes’ women suffer from various symptoms initially such as hot flushes, sweating, insomnia, headache, osteoporosis etc. Ovarian estrogen begins to decline 1 or 2 years before menopause and becomes stable 2 years after menopause. Direct effects of estrogen influences brain function through effects on vasculature and immune system. Estrogen actions are potentially relevant to cognitive changes occurring after menopause. Estrogen enhances neurite growth, neurogenesis, long term potentiation and episodic memory and has influence on neurotransmitter. Functional brain imaging studies demonstrate that estrogen modulates neural activity during performance of cognitive task. Around the time of menopausal transition, many women report problem with memory (episodic memory), attention n, sleep problems, poor concentration, difficulty recalling an acquaintances name, leading to anxiety and depression. Factors such as mood, stress, physical heath may also contribute to memory symptoms.²

Cognitive therapy states that thoughts, feelings and behavior are all connected and that individual can move towards overcoming difficulties and meeting their goals by identifying and changing unhelpful or inaccurate thinking, problematic behavior and distressing emotional responses. This also involves the individual testing and modifying beliefs, identifying distorted thinking, relating to others, relaxation and many techniques to improves your overall performance.

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Cognitive therapy is a systematic process utilizing leisure activities and other interesting activities as interventions to address the needs of individuals with cognitive impairments as a means of recovery and well-being.

Study regarding the effects of cognitive therapy on memory, attention, anxiety and depression has not been conducted. We wanted to study its effect in postmenopausal women in whom attention and memory is reduced and signs of depression and anxiety are present.

Materials and Methodology

An approval for the study was obtained from the Protocol committee and the Institutional Ethical Committee of KIMSDU. A study was conducted for a duration of 6 months which was conducted in and around Karad. Individuals were approached and those fulfilling the inclusive criteria were selected.

Subjects

All participants were selected by Convenient sampling method. Each of them was selected according to the inclusion criteria. Female participants were included. Individuals having cognitive changes and willing to participate, between age group 45-65 years were included. Women having any other neurological deficit, any hormonal therapy, suffering from depression and women who had surgical menopause were excluded from the study. 100 participants were screened for the study, 50 individuals who fulfilled the inclusion and exclusion criteria was selected. The procedure was explained and written informed consent were taken from those willing to participate. A session was held in Karad, where data collection was done and exercises were performed for 4 days per week for 4 weeks. Demographic information of the subjects was collected. Primary outcome measure used was Mini mental scale, Mood scale, Hamilton depression rating scale. All the score was taken before and after the treatment.

If the individuals were explained about the purpose of the study. They were informed about the procedure. Each participant was assessed and the scores were calculated and the Data was recorded for the individual participant.

Statistical Analysis

Statistical analysis was done using unpaired t test. The analysis was performed using the software Instat. Arithmetic means & standard deviation was calculated for each outcome measure and Arithmetic mean was derived from adding all the values together and dividing the total number of values. MS Excel was used for drawing various graphs with given frequencies and the various percentages that were calculated with the software.

Standard deviation (SD) was calculated according to the following formula:

\[
SD = \sqrt{\frac{1}{N} \sum (X - \mu)^2}
\]

Results

The results showed that there is extremely significant difference in cognitive changes in postmenopausal women. (p value >0.0001)

Discussion

The current study was aimed to find the effect of cognitive therapy in postmenopausal women with memory, attention, anxiety and depression difficulty when given for 4 weeks. Total 100 post-menopausal women were screened and 50 individuals having cognitive changes and fulfilling the inclusion and exclusion criteria were selected. Objectives of this study was to find whether there is any effect of cognitive therapy on postmenopausal women with memory, attention, anxiety and depression difficulty; and if yes, how did it affect the daily activities.

A study by Henderson VW compared various age groups with cognitive changes due to estrogen activity. He reported the changes that occur in midlife and later in lifetime. There was significant decline in cognitive changes after 80-85 years and drug therapy showed no improvement in the cognitive changes. In this article we have selected the age group between 45-65 years as cognitive changes are seen more in this age group.

A recently reported randomized trial of selective estrogen receptor modulator reported on cognition in postmenopausal women revealed no significant difference, although the women who received raloxifene tended to have a slightly lower risk of decline in cognitive therapy as measured by testes of verbal memory and attention. Raloxifene and estrogen may not affect overall cognition but may protect against the development of
cognitive impairment.  

Previously conducted research on estrogen activity on the structure and function of brain areas was known to be involved in memory and attention. Controlled study of administration of estrogen to postmenopausal women have found that estrogen enhances verbal memory and maintain the ability to learn new material.  

Previously conducted research on HRT activity concluded that there is good evidence that both HRT and ERT do not prevent cognitive decline in older postmenopausal women when given as short term or long-term therapy. It remains to be determined whether factors such as younger age, type of menopause, type of treatment, mode of delivery, and dosage have any positive effects at clinically relevant level. The main cause of change in cognitive function is associated by the activity of estrogen to protect and maintain brain structures. Thus, it is biologically plausible that maintaining high levels of estrogen in postmenopausal women could be protective against cognitive decline.  

Previously conducted study on cognitive therapy in depression reviewed that cognitive therapy is more effective than behavior therapy or any other forms of psychotherapy addition, it appears that cognitive therapy is independent of the length of the therapy, because the average length of therapy in this study was only 14.9 weeks. It is not clear, however, to what extent the process of cognitive therapy achieves changes in depressive types or to what extent cognitive therapy achieves its therapeutic outcomes through the specific modification of cognitive distortions or depressogenic assumptions.  

A study conducted on cognitive rehabilitation for attention deficits following stroke stated that cognitive therapy can improve alertness and sustained attention but there is no evidence that it helps people to do daily activities without help after stroke. Attention problems can occur following stroke, although there is spontaneous recovery with cognitive therapy. The exact mechanism is still unknown and more study needs to be done.  

There are cognitive changes seen even after HRT, ERT drug treatment and patients with stroke who are not under medications have showed significantly improved results by cognitive therapy. Hence, cognitive therapy may also effective in post-menopausal women. From the above-mentioned effects of cognitive therapy on memory, attention, anxiety and depression. It may significantly improve cognitive functions in postmenopausal women.  

**Conclusion**

The present study provided evidence to support the use of cognitive therapy in reducing the depression and anxiety and increasing the memory and attention in post-menopausal women. Thus, the Alternate hypothesis is proved.

**Financial Support and Sponsorship:** This study was funded by Krishna Institute of Medical Sciences Deemed to Be University, Karad.

**Conflict of Interest:** There were no conflicts of interest.

**Ethical Clearance Certificate:** Taken

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Effect of Meditative Movement Exercises with Breath Control on Depression in Nulliparous Women

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Abstract

Background: The prevalence of nulliparous women in India is 66% out of 8 million married women. In nulliparous women depression is the most common factor due to various other factors as single mother, or are not able to conceive, low income earners and those in unsupportive social situations. So as to reduce the depressive symptoms or thoughts we can teach them a set of meditative movement exercises specially in women who are nulliparous.

Objective: To study the effect of meditative movement exercises with breath control on depression in nulliparous women.

Methodology: The study was conducted in and around Karad, Maharashtra, and was carried over a period of 12 weeks. Study was conducted using sample size of 22 nulliparous women with minimal to moderately severe depression. The inclusion criteria was age group of 25 – 35 years females with exclusion criteria as history of high blood pressure, chronic medical illness and major psychiatric problem.

Results: Statistical analysis for effect of meditative movement exercise on depression in nulliparous women was extremely significant (P value=0.0001). It was extremely significant during pre-test as well as post-test. So there is improvement in the depression score after teaching the exercises to the individuals.

Conclusion: From this study, it can be concluded that the individuals showed improvement in depression levels after teaching them the meditative movement exercises.

Key words: depression, nulliparous women, breath control, age group 25-35 years, meditative movement exercises.

Introduction

Nulliparous means a women who has never given birth either by choice or for any other reason. This term is also used in case of women who give birth to a still born baby, or a baby who is not able to survive outside the mother’s womb. Globally 75% of women are nulliparous and have problems in conceiving. In India, 66% of 8 million married women are nulliparous. Among married young women aged from 20 to 24 years about 9.1 million are nulliparous. The main causes which lead to nulliparity in women can be ovulation problems caused due to polycystic ovarian syndrome, due to older age as in this period the female may face various problems as high blood pressure or gestational diabetes, nulliparity can also be caused due to endometriosis as they block the fallopian tube cause disrupt implantation and impact the egg quality, the other cause can be overweight due to inactive lifestyle or due to any eating disorder as anorexia or bulimia, due to tubal issues caused mainly due to STD and due to uterine abnormalities i.e. due to fibroids which develop within the wall of uterus.

Women who can’t conceive often experience distressing emotions as shock, depression, anger and frustration as well as loss of self-esteem, self-confidence. They may also avoid social interaction with family and friends. Depressive disorders have become a widespread health concern throughout the world. The worldwide prevalence of depression and anxiety has been estimated at 10.4%. Some of the reasons nulliparous women struggle with depression include stress due to pressure on the women to get pregnant,
medical conditions such as PCOS may also increase the risk of depression, the emotional and physical challenges of treatment for infertility. It is not unusual to feel depressed occasionally. However, when these feelings persist over time and affect a person’s quality of life, they may be experiencing depression. Some symptoms of depression are loss of interest in most activities, sleeping too much or too little, having low energy, feeling worthless, trouble in concentrating or thinking. There are no studies being conducted on whether depression can cause infertility in women though some studies have just found a correlation between depression and increased rates of infertility. Also, depression may lead to lifestyle habits that can negatively impact fertility. For example, depression often causes overeating, being overweight or underweight which can cause infertility in women. Another factor is women who are depressed may often smoke or drink which can also cause problems to conceive. For measuring the depression level of the subject PHQ-9 questionnaire was used, which is basically used in multipurpose screening, diagnosing, and measuring the severity of depression.

To reduce the nulliparity and depression there are various exercises. Studies show that physical activity reduces depression and anxiety. Exercise also helps to reduce the risk for physical comorbidities that occur with depression. Studies show that moderate regular exercise can improve fertility and the chance of conceiving increases. Studies has shown that vigorous exercise reduces the risk of ovulation and that moderate exercise decrease the risk of miscarriage and increase the chance of conceiving. While some studies show that low intensity exercises are helpful in reducing nulliparity as meditation, yoga etc. whereas high intensity exercises should be avoided as it may reduce the fertility. So, teaching low intensity exercises is helpful in women who can’t conceive. There are various ways as relaxation, meditative movement exercises as well as yoga.

Payne and Godreau (2013) used the term Meditative Movement for forms of physical exercise which concentrates towards the bodily sensations, including proprioceptive, interoceptive, and kinesthetic sensations. The general overall purpose of meditative movement exercise is to increase vitality, balance circulation and to harmonize body-mind relationship. Meditative movement exercises has shown a positive effect on depression. These cause change in levels of cytokines and growth hormone. As this exercise increases the body temperature and at the same time the temperature of brain increases which results in feeling of general relaxation. It also prevents various disorders and improve the functioning of the body which helps in reducing the depression in these women. It also increases the appetite and quality of sleep.

**Material and Methodology**

An approval for the study was obtained from the Protocol committee and the Institutional Ethical Committee of KIMSDU. An Experimental study was conducted for duration of 6 months at Physiotherapy department of Krishna college of Physiotherapy. Individuals were approached and those fulfilling the inclusive criteria were selected. This study included 22 individuals with depression according to inclusion and exclusion criteria. These individuals were given a PHQ-9 questionnaire prior to check the level of depression they had on minimal to moderately severe depression scale. Then according to the level of depression and the inclusion criteria individuals were selected and were taught all the meditative movement exercises. These exercises are performed in sets lasting about 20min per day. These are followed in following ways-


**Outcome Measures:**

The outcome measure used for this study was PHQ-9 Questionnaire.

This questionnaire aims at evaluating the degree of depression severity. It is a multipurpose questionnaire used for screening, diagnosing, monitoring and measuring the severity of depression.

**Statistical Analysis**

Statistical analysis of the recorded data was done by using the software SPSS version20.

22 subjects were successfully completed by giving them the questionnaire prior and after teaching them exercises. The result showed that mean of the pre-test score of depression is 13.22 and SD is 1.744. The mean value of post test score of depression is 7.318 and SD is 1.524.
### Result

The statistical analysis for the pre-test depression score shows significance and after the exercises given to the subjects the post-test depression score is extremely significant. There was improvement in the depression score after giving the meditative movement exercises.

### Discussion

Depression has been a major widespread health concern. The worldwide prevalence of depression has been estimated at 10.4% (Andrews et al 2000). During pregnancy, depression affects 10-50% of women with the incidence being higher in low socioeconomic status. Many studies have been conducted on effect of aerobic exercise training on depression in nulliparous women but there are very few studies on this category of exercises. The present clinical trial was conducted to compare the effectiveness of meditative movement exercises with breath control on depression in nulliparous women. Results of this study were focused on improving or reducing the depression in nulliparous women by advising them set of meditative movement exercises.

In this study, 22 nulliparous women suffering from minimal to moderate depression were selected between the age group of 25-35 years. They were selected based on the inclusion criterion. They were given a PHQ-9 questionnaire prior, to check the level of depression before advising them the meditative movement exercises. After filling the questionnaire from the subjects they were checked on the basis of having minimal or moderately severe depression according to that they were given a set of meditative movement exercises. They were advised to do these exercises daily for 15 to 20 minutes. After giving them the exercises, the subjects were assessed again after 3 months to check the effect of those exercises on depression level. The percentage of depression before the test is 59% moderate depression, 35% moderately severe depression, 5%mild depression and 1% minimal depression and after teaching them the exercises the percentage of depression is 71% minimal depression, 23% mild depression, 5% moderate and 1% moderately severe depression.

Our study showed that there were considerable changes with significant difference seen in the level of
depression in the nulliparous women, as we analyzed that the changes in the PHQ-9 questionnaire for screening the depression level, before and after incorporating meditative movement exercises.

In a study done by Angelo Fernando Robledo-Colonia, they showed effect of aerobic exercise training on depression in nulliparous women where they had an experimental group to perform 60-min exercise classes per week, starting from week 16 and 20 of gestation and continuing for 3 months. Sessions consisted of walking, stretching, aerobic exercises with relaxation.

In another study done by Eun Sun Ji, RN, they showed effect of Qi exercises on maternal/fetal interaction and maternal well-being during pregnancy where they selected 18 weeks pregnant women and gave them Qi exercises to perform twice a week for 90 minutes for 12 weeks. The results showed that Qi exercises lowered maternal depressive symptoms and improved the physical health of the mother.

This study showed has a positive influence among the nulliparous women with depression. And results were found to be statistically significant by the use of PHQ-9 questionnaire.

**Conclusion**

After analyzing the data, it was found that there is a significant effect of meditative movement exercises with breath control on depression in nulliparous women.

**Abbreviations:**

PHQ-9: Patient Health Questionnaire

MME: Meditative movement exercises

**Conflict of Interest:** There is no conflict of interest concerning the interest of study.

**Source of Funding:** This study is self-funded.

**Ethical Clearance:** The study was approved by institutional ethical committee of KIMSDU.

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To Assess Dentist Knowledge About Lipid Treatment of Local Anesthetic Systemic Toxicity

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Abstract

Background: The present study was conducted to assess dentist knowledge about lipid treatment of local anesthetic systemic toxicity.

Materials & Method: This study was conducted on 260 dentists of both genders. All were provided with a questionnaire. Information regarding LAST, signs of LAST they encountered and treatments for LAST, the length of professional experience, training on local anesthesia, LAs used most often, side effects seen most often were recorded.

Results: Out of 260 dentists, 140 were male and 120 were female. 0-5 years experience was seen in 20, 5-10 in 70, 10-15 years in 50 and >15 years in 120 dentists. The difference was significant (P< 0.05). 62% had no idea of lipid treatment, 23% had heard but did not have enough knowledge of it, 13% had read an article about lipid treatment and 2% knew how to use lipid treatment. The difference was significant (P< 0.05). 85% had never seen LA toxicity, 12% had not use lipid treatment in local anesthesia toxicity and 3% had use lipid treatment in local anesthesia toxicity. The difference was significant (P< 0.05).

Conclusion: Authors suggested that dentists must be aware of LA toxicity and knowledge of how to treat it.

Key words: Knowledge, LAST, local anesthetic

Introduction

An ideal local anesthetics should be potent, reversible, low cost, stable, and easy to metabolize and emit. Furthermore, it should have rapid onset action, suitable duration of effect, and good tissue penetration. Another one of the important qualifications is that there should be no adverse effect, neither local nor systemic.¹ Complications due to local anesthesia can be divided into three major areas; complications associated with vasoconstrictor, needle, and absorption of local anesthetics. Complications associated with the added vasoconstrictor include elevated blood pressure and increased heart rate. Another complications associated with the needle include syncope, hematoma, pain, edema, infection, paresthesia, nerve paralysis, and breakdown of needle or cartridge. Absorption of the local anesthetics includes local reaction, idiosyncrasy, toxicity, and allergy or anaphylactic reaction.² All amidetype LAs can cause local anesthetic systemic toxicity (LAST), and in addition, risk of cardiovascular system (CVS) and central nervous system (CNS) toxicity is still a major problem. LAST is a very serious condition that can cause death.³ In LAST, blood levels of LAs exceed the maximum dose, usually as a result of multiple injections of the anesthetic or accidental injection into a vein. If the level of LA in the blood reaches an individual’s maximum dose, LAST may occur. LAs are the drugs most often used by dentists, and although dentists may encounter few adverse events, this does not change the fact that LAST can occur. Overdose, anatomy, and other patientspecific factors can contribute to LAST. Intravascular injection

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has been reported in 15.3% of cases when the inferior alveolar nerve block is targeted. The present study was conducted to assess dentist knowledge about lipid treatment of local anesthetic systemic toxicity.

**Material & Method**

This study was conducted in the department of Oral Surgery. It comprised of 260 dentists of both genders. All were informed regarding the study and written consent was obtained. Ethical clearance was obtained prior to the study.

Data such as name, age, gender etc. was recorded. All were provided with a questionnaire. Information regarding LAST, signs of LAST they encountered and treatments for LAST, the length of professional experience, training on local anesthesia, LAs used most often, side effects seen most often were recorded. Results were subjected to statistics. P value less than 0.05 was considered significant.

**Table I Distribution of patients**

<table>
<thead>
<tr>
<th>Gender</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>140</td>
<td>120</td>
</tr>
</tbody>
</table>

Table I shows that out of 260 dentists, 140 were male and 120 were female.

**Table II Experience of dentists**

<table>
<thead>
<tr>
<th>Experience (Years)</th>
<th>Number</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-5</td>
<td>20</td>
<td>0.01</td>
</tr>
<tr>
<td>5-10</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>10-15</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>&gt;15</td>
<td>120</td>
<td></td>
</tr>
</tbody>
</table>

Table II shows that 0-5 years experience was seen in 20, 5-10 in 70, 10-15 years in 50 and >15 years in 120 dentists. The difference was significant (P< 0.05).

**Table III Do you know lipid treatment in local anesthesia toxicity?**

<table>
<thead>
<tr>
<th>Answer</th>
<th>Percentage</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No idea of lipid treatment</td>
<td>62%</td>
<td></td>
</tr>
<tr>
<td>Had heard but did not have enough knowledge of it</td>
<td>23%</td>
<td>0.02</td>
</tr>
<tr>
<td>Knew how to use lipid treatment</td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td>Had read an article about lipid treatment</td>
<td>13%</td>
<td></td>
</tr>
</tbody>
</table>

Table III shows that 62% had no idea of lipid treatment, 23% had heard but did not have enough knowledge of it, 13% had read an article about lipid treatment and 2% knew how to use lipid treatment. The difference was significant (P< 0.05).
Graph I: Have you ever use lipid treatment in local anesthesia toxicity?

Graph I shows that 85% had never seen LA toxicity, 12% had not use lipid treatment in local anesthesia toxicity and 3% had use lipid treatment in local anesthesia toxicity. The difference was significant (P< 0.05).

Discussion

LAST refers to the complication particularly affecting the central nervous system (CNS) and cardiovascular system (CVS) due to the overdose of local anesthetics. LAST is a life-threatening complication and reportedly occurs in 0.03% people.5 To elaborate, the order of incidence of complications from highest to lowest based on the type of block is penile, local infiltration, neuraxial, upper extremity, paravertebral, lower extremity, head and neck, topical, transversus abdominis plane, and intravenous block. Penile blocks are most commonly associated with complications, because of the large distribution of blood vessels. This is followed by local infiltration, which is commonly used in dentistry.6 The order of incidence of complications from highest to lowest according to the local anesthetics used is bupivacaine, lidocaine, ropivacaine, mixture local anesthetics, levobupivacaine, chloroprocaine, and articaine. Lidocaine, the most commonly used anesthetic in dentistry, accounted for approximately 25% of complications reported. The high incidence of complications of lidocaine, which is comparatively safe, could be due to its high frequency of use.7 The present study was conducted to assess dentist knowledge about lipid treatment of local anesthetic systemic toxicity.

In this study, out of 260 dentists, 140 were male and 120 were female. 0-5 years experience was seen in 20, 5-10 years in 70, 10-15 years in 50 and >15 years in 120 dentists. Vijayalakshmi et al8 have described the toxicity caused by overdose of local anesthetics during dental treatments and the pharmacokinetic progression, and reported the importance of drug selection and safety levels of the drugs.

We found that 62% had no idea of lipid treatment, 23% had heard but did not have enough knowledge of it, 13% had read an article about lipid treatment and 2% knew how to use lipid treatment. 85% had never seen LA toxicity, 12% had not use lipid treatment in local anesthesia toxicity and 3% had use lipid treatment in local anesthesia toxicity.

Basaranoglu et al9 administered 600 questionnaires that asked about the frequency with which dentists encountered LAST and the symptoms of LAST and its treatment, especially with lipids. The results showed that 520 (86.66%) respondents had never seen LAST, and 404 (67.3%) had no idea about lipid treatment. In addition, 128 (21.3%) had heard about lipid treatment but had inadequate knowledge of it and 59 (9.8%) had read an article about lipid treatment, but only 9 (1.5%) knew how to use lipid treatment. Finally, 80 (13.33%)
participants had seen LAST but had used a treatment other than lipids.

Guzmán et al.\textsuperscript{10} in their study interviewed anesthetists at hospitals about their knowledge and use of lipid therapy for LAST. Results showed that 50% of the anesthetists knew about lipid therapy and had adequate information to use it correctly. However, it also found that they did not know how to obtain lipids and that lipid solutions were not kept in locations where local anesthesia was administered.

**Conclusion**

Authors suggested that dentists must be aware of LA toxicity and knowledge of how to treat it.

**Conflicts of Interest:** The authors declare that there is no conflict of interest regarding the publication of this paper.

**Source of Funding:** Self

**Ethical Clearance:** Ethical clearance has been taken from Institutional Ethical Committee

**References**


Assessment of Medico- Legal Awareness of Practicing Obstetricians and Gynecologists

Ajay V Patil¹, Rajendra Bangal²

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Abstract

**Background:** In the care of suffering the medical professionals need scientific knowledge, technical skill, moral understanding of profession and awareness about the relevant laws of the land. The present study was conducted to assess medico- legal awareness of practicing obstetricians and gynecologists.

**Materials & Method:** The present study was conducted on 156 gynecologist. All were provided with standard questionnaire consisting of ten questions was given to them and their response was collected.

**Results:** Age group 30-45 years had 30, 35-40 years had 45, 40-45 years had 50 and >45 years had 31 subjects. The difference was significant (P< 0.05). Questionnaire used was promptness in examination of victim of sexual assault, procedure related to Death Certification in MLCs, evidence Preservation in rape cases, awareness of Laws related to Obst.& Gynac Practice, real Causes of the Negligence Complaints, Awareness Regarding the provisions of PCPNDT Act and awareness about need of examining genuine MLCs. Most of the subjects was aware. The difference was significant (P< 0.05).

**Conclusion:** Most of the gynecologist had sufficient awareness regarding medico- legal issues.

**Key words:** Gynecology, Medicolegal, victims.

**Introduction**

No greater opportunity, no greater responsibility, no greater obligation can fall to any other human being than to become a medical professional. In the care of suffering the medical professionals need scientific knowledge, technical skill, moral understanding of profession and awareness about the relevant laws of the land. Primarily doctors but also on other ancillary and administrative staff of the hospital have several ethical and legal obligations in the performance of their duties.

It is important therefore, that every concerned person of the hospital, related with treatment and care of the patients must understand the nature of obligations and thus fulfills these obligations to the best of their ability, to maintain nobility of medical profession.

The obstetricians and gynecologists are one of the important specialties of medical faculty. Increase in life expectancy, decreasing number of female births and reduction in family size cast a significant quantum of accountability on these “doctors. They are working day and night for the benefit of patients. They are also very helpful with the Mother and Child Health Programme, Family Planning Mission, etc. of Government of India. The obstetricians and gynecologists are also helpful to society at large for their work for crime against woman, in the form of examination of victim of sexual abuse, the PC & PNDT act, Maternal Mortality. The present study was conducted to assess medico- legal awareness of practicing obstetricians and gynecologists.

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**Materials & Method**

The present study was conducted in the department of Forensic Medicine and Department of Gynaecology.
It consisted of 156 gynecologists. All subjects were informed about the study and written consent was taken. Ethical clearance was obtained prior to the study.

Data such as name, age, sex etc. was recorded in performa. All were provided with standard questionnaire consisting of ten questions was given to them and their response was collected. The questions in the questionnaire were mainly in relation to promptness, procedural awareness, awareness of laws, insight about causes of negligence & their experience with agencies of crime investigation & administration of justice. Results thus obtained were studied. P value <0.05 was considered significant.

**Results**

**Table I Age wise distribution**

<table>
<thead>
<tr>
<th>Age group (Years)</th>
<th>Number</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-35</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>35-40</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>40-45</td>
<td>50</td>
<td>0.01</td>
</tr>
<tr>
<td>&gt;45</td>
<td>31</td>
<td></td>
</tr>
</tbody>
</table>

Table I shows that age group 30-45 years had 30, 35-40 years had 45, 40-45 years had 50 and >45 years had 31 subjects. The difference was significant (P< 0.05).

**Table II Assessment of awareness**

<table>
<thead>
<tr>
<th>Questions</th>
<th>Aware</th>
<th>Partly aware</th>
<th>Not aware</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Promptness in examination of victim of sexual assault</td>
<td>80</td>
<td>36</td>
<td>40</td>
<td>0.05</td>
</tr>
<tr>
<td>Procedure related to Death Certification in MLCs</td>
<td>72</td>
<td>40</td>
<td>44</td>
<td>0.02</td>
</tr>
<tr>
<td>Evidence Preservation in rape cases</td>
<td>57</td>
<td>60</td>
<td>39</td>
<td>0.01</td>
</tr>
<tr>
<td>Awareness of Laws related to Obst.&amp; Gynac Practice</td>
<td>70</td>
<td>50</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Real Causes of the Negligence Complaints</td>
<td>60</td>
<td>46</td>
<td>50</td>
<td>0.24</td>
</tr>
<tr>
<td>Awareness Regarding the provisions of PCPNDT Act</td>
<td>56</td>
<td>58</td>
<td>42</td>
<td>0.76</td>
</tr>
<tr>
<td>Awareness about need of examining genuine MLCs</td>
<td>65</td>
<td>55</td>
<td>36</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Table II shows that questionnaire used was promptness in examination of victim of sexual assault, procedure related to Death Certification in MLCs, evidence Preservation in rape cases, awareness of Laws related to Obst.& Gynac Practice, real Causes of the Negligence Complaints, Awareness Regarding the provisions of PCPNDT Act and awareness about need of examining genuine MLCs. Most of the subjects was aware. The difference was significant (P< 0.05).

**Discussion**

There is increased use of internet, electronic and print media there is an increase in awareness among public on the subject of ethical conduct of medical practitioners. Hence there are more cases against doctors, which is an issue of immediate concern to the medical fraternity. To keep away from this, doctors need to make acquainted themselves with laws and regulations that concern their
It is a known fact that adverse outcomes to the patients in the course of healthcare management are frequent day by day and this is not without the danger of professional risk to this group of practitioners. These risks range, from failure to attend patient, examination and diagnosis at one end to specific investigative procedures and complicated operations and other. Same is the story in relation to the medicolegal management of cases of violence against woman. It is interesting to note that the social, medical and legal requirements in both these situations are qualitatively almost similar. The medical science is advancing and law is encroaching in almost every area of medical practice. It also cannot be ignored that society deserves the right to nourish great expectations from the advances in modern medicine and legal provisions aimed at their welfare. The present study was conducted to assess medico-legal awareness of practicing obstetricians and gynecologists.

In this study, age group 30-45 years had 30, 35-40 years had 45, 40-45 years had 50 and >45 years had 31 subjects. Kachare et al conducted a study to assess medico legal knowledge and awareness in interns and post graduate students. Total 200 students were included in our study. We found that 84 (70%) interns and 32 (40%) post graduate students had no proper knowledge in handling medico legal cases independently. 55 (45.83%) interns and 63 (78.75%) post graduate students were of opinion that the present UG teaching is not sufficient for them to tackle medico legal cases. Only 36 (30%) interns and 33 (41.25%) post graduate students were aware about the preservatives that are generally used for chemicals analysis of organs and 25 (20.83%) interns and 65 (81.25%) post graduate students were aware of Consumer Protection Act.

In present study, questionnaire used was promptness in examination of victim of sexual assault, procedure related to Death Certification in MLCs, evidence Preservation in rape cases, awareness of Laws related to Obst.& Gynac Practice, real Causes of the Negligence Complaints, Awareness Regarding the provisions of PCPNDT Act and awareness about need of examining genuine MLCs. Most of the subjects was aware.

Giri et al found that awareness status regarding different medico-legal case management like medico-legal cases, Promptness in examination of victim of sexual assaults, Death certification in MLC, Evidence Preservation in MLC, Medico-legal severity of injury, Importance of Identity in MLC, Criminal Abortion Cases, Awareness of Law related to medical Practice, Real causes of Negligence complaints, Awareness regarding the provisions of PCPNDT Act, Importance of good medical record and Rights of the patients etc. 33.69% were aware, 31.02% were partly aware and 24.93% were not aware and 10.36% has given no response in related subject matter or issues.

### Conclusion

Most of the gynecologist had sufficient awareness regarding medico-legal issues.

### Conflicts of Interest:
The authors declare that there is no conflict of interest regarding the publication of this paper.

### Source of Funding:
Self

### Ethical Clearance:
Ethical clearance has been taken from Institutional Ethical Committee

### References


10. Giri PA, Chavan KD, Phalke DB. Knowledge about medicolegal problems amongst the interns and resident from rural tertiary care teaching hospital in western Maharashtra-A cross sectional study. Indian journal of Forensic Medicine and Pathology,
Pattern of Cranio-Cerebral Injuries at a Tertiary Care Centre – A Retrospective Study

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Abstract

Head injuries are accounting for most serious injuries in terms of morbidity and mortality1. The present study was carried out at a tertiary care hospital, Maharashtra for duration of three years. This study was conducted on 173 cases, which were directly brought to mortuary for postmortem examination from the site of incidence. The objective of this study is to find out the pattern of skull fractures irrespective of cause of injury. Linear fracture was found to be more common among the total no of cases. In the present study it was observed that skull fractures are more commonly seen in assault cases. In this study it is observed that extradural hemorrhage is most commonly associated with motor cycle riders. It has been also noticed that younger age group (21-30 years) are most commonly effected age group. In this present study it is noticed that abrasions are the most common type of injuries associated with skull fractures.

Key words: Cranio-cerebral injuries, Skull fractures, Hemorrhages and autopsy.

Introduction

Cranio-cerebral injuries accounts for the most serious injuries in terms of morbidity and mortality.1 Cause of cranio-cerebral injuries may be assault, RTA, fall from height and even fall on ground.1 Head is the most common part injured in road traffic accident as it is the most prominent and vulnerable part of human body by virtue of its situation and to sustain serious and fatal injuries owing to the great risk of striking the head.2 Depending upon the severity of violence involved, cranio-cerebral injuries can be associated with injury to the scalp, skull, intracranial hemorrhages and injury to brain tissue.2 Cranio-cerebral injuries are a major public health problem and have already attained epidemic proportion in India. As a result cranio-cerebral trauma places a huge financial and psychological burden upon the society.3

Material and Method

The present study was carried out at CPR hospital of RCSM GMC Kolhapur, for period of three years. This study was done on 173 cases, which were directly brought to mortuary for postmortem examination from site, irrespective of cause of injury. Hospitalized and operated cases were excluded from the present study.

The present study has been carried out after obtaining the ethical clearance and consent from the relatives to collect the relevant information.

Meticulous postmortem examination was done, skull and brain was specifically studied for the presence of fracture, hematoma, hemorrhage and injury to the brain tissue. To study the fractures and other injuries, magnifying lenses and measuring tape were used. Detailed study of police inquest and police record was done.

All data was collected and analyzed.

Results

The results of the present study which was carried on 173 cases at CPR hospital of RCSM GMC Kolhapur, for period of three years, which were directly brought to mortuary for postmortem examination from site,

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irrespective of cause of injury are as follows.

In the present study we found that skull fractures are more commonly seen in motorcycle riders cases i.e. 56 cases (32.36%) followed by assault i.e. 52 cases (30.05%), least commonly seen in fall from height i.e. 7 cases (4%).

<table>
<thead>
<tr>
<th>Case</th>
<th>No of Cases</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motor Cycle Rider</td>
<td>56</td>
<td>32.36 %</td>
</tr>
<tr>
<td>Assault</td>
<td>52</td>
<td>30.05 %</td>
</tr>
<tr>
<td>Pillion Rider</td>
<td>41</td>
<td>23.69 %</td>
</tr>
<tr>
<td>Four Wheeler Accident</td>
<td>09</td>
<td>5.20 %</td>
</tr>
<tr>
<td>Railway Accident</td>
<td>08</td>
<td>4.60 %</td>
</tr>
<tr>
<td>Fall From Height</td>
<td>07</td>
<td>4.10 %</td>
</tr>
<tr>
<td>Total</td>
<td>173</td>
<td>100 %</td>
</tr>
</tbody>
</table>

Table 1: Pattern of skull fractures according to the type of case.

Most common type of fracture seen is linear fracture i.e. 72 cases (38.92%) followed by depressed fracture i.e. 59 cases (31.89%), and least common type of fracture seen is ring fracture i.e. 1 case (0.54%). Sutural fracture, gutter fracture and ponds fracture are not seen in any cases of this study.

<table>
<thead>
<tr>
<th>Type of Fracture</th>
<th>No of Cases</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear</td>
<td>72</td>
<td>38.92 %</td>
</tr>
<tr>
<td>Depressed</td>
<td>59</td>
<td>31.89 %</td>
</tr>
<tr>
<td>Communitened</td>
<td>51</td>
<td>27.57 %</td>
</tr>
<tr>
<td>Signature</td>
<td>2</td>
<td>1.08 %</td>
</tr>
<tr>
<td>Ring</td>
<td>1</td>
<td>0.54 %</td>
</tr>
<tr>
<td>Sutural</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gutter</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ponds</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>185</td>
<td>100 %</td>
</tr>
</tbody>
</table>

Table 2: Pattern of type of skull fracture.

In this present study we found that the most common type of Intracranial hemorrhage is extradural hemorrhage; i.e. 121 cases (40.88%), followed by subdural hemorrhage i.e. 102 cases (34.46%). least common type of intracranial hemorrhage is intra cerebral i.e. 12 cases (4.06%) and is always seen with one or the other type of intracranial hemorrhage.

<table>
<thead>
<tr>
<th>Type of Cerebral Hemrrhage</th>
<th>No of Cases</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extradural</td>
<td>121</td>
<td>40.88 %</td>
</tr>
<tr>
<td>Subdural</td>
<td>102</td>
<td>34.46 %</td>
</tr>
<tr>
<td>Subabrachinodid</td>
<td>61</td>
<td>20.60 %</td>
</tr>
<tr>
<td>Intracerebeller</td>
<td>12</td>
<td>4.06 %</td>
</tr>
<tr>
<td>Total</td>
<td>296</td>
<td>100 %</td>
</tr>
</tbody>
</table>

Table 3: Pattern of intracranial hemorrhage associated with skull fracture.

In this study we also found that most common type of injury associated with skull fractures is abrasion i.e. 156 cases (34.36%), followed by contused lacerated wounds i.e. 131 cases (28.85%) and least common type of injury seen is fire arm injury i.e. 1 case (0.22%). And most of the cases, these injuries are seen along with the one or the other type of injury.

<table>
<thead>
<tr>
<th>Oter Injuries</th>
<th>No of Cases</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abortion</td>
<td>156</td>
<td>34.36 %</td>
</tr>
<tr>
<td>Clw</td>
<td>131</td>
<td>28.85 %</td>
</tr>
<tr>
<td>Fracture</td>
<td>108</td>
<td>23.79 %</td>
</tr>
<tr>
<td>Crush Injuries</td>
<td>31</td>
<td>6.83 %</td>
</tr>
<tr>
<td>Incised Wound</td>
<td>21</td>
<td>4.62 %</td>
</tr>
<tr>
<td>Stab Injury</td>
<td>6</td>
<td>1.32 %</td>
</tr>
<tr>
<td>Fire Arm Injury</td>
<td>1</td>
<td>0.22 %</td>
</tr>
<tr>
<td>Total</td>
<td>454</td>
<td>100 %</td>
</tr>
</tbody>
</table>

Table 4: Pattern of type of injury associated with skull fractures.

In this present study we also found that most commonly effected age group of victims is 21-30 years i.e. 52 cases (30%), followed by 31-40 years i.e. 51 cases
least commonly effected age group is 61-70 years i.e. only 1 case (1%). It is also seen that males (84.4 %) are most commonly affected as compare to females (15.5 %).

Table 5: Pattern of skull fractures according to the age group and sex.

<table>
<thead>
<tr>
<th>Age Group</th>
<th>MALE</th>
<th>FEMALE</th>
<th>TOTAL</th>
<th>PERCENTAGE %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10 years</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>3%</td>
</tr>
<tr>
<td>11-20 Years</td>
<td>26</td>
<td>6</td>
<td>32</td>
<td>18%</td>
</tr>
<tr>
<td>21-30 Years</td>
<td>44</td>
<td>8</td>
<td>52</td>
<td>30 %</td>
</tr>
<tr>
<td>31-40 Years</td>
<td>47</td>
<td>4</td>
<td>51</td>
<td>29 %</td>
</tr>
<tr>
<td>41-50 Years</td>
<td>19</td>
<td>5</td>
<td>24</td>
<td>14 %</td>
</tr>
<tr>
<td>51-60 Years</td>
<td>6</td>
<td>1</td>
<td>7</td>
<td>5 %</td>
</tr>
<tr>
<td>&gt;60 Years</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1 %</td>
</tr>
<tr>
<td>Total</td>
<td>146</td>
<td>27</td>
<td>173</td>
<td>100 %</td>
</tr>
</tbody>
</table>

Discussion

The present study was carried out at CPR hospital of RCSM GMC Kolhapur, for period of three years. This study was done on 173 cases, which were directly brought to mortuary for postmortem examination from site, irrespective of cause of injury. This study shows that the skull fractures are more common in motorcycle riders cases i.e. 56 cases (32.36%) followed by assault i.e. 52 cases (30.05%). Similar findings were observed by Kirti jaiswal et al.4 The most common type of fracture seen is linear (41%) followed by depressed fracture. similar findings are observed by Sreekanth S Nair et al.5 The most common intracranial hemorrhage seen is extradural hemorrhage (69%), followed by Subdural hemorrhage (58%), similar findings were observed by Rahman MA et al.6 The most commonly seen type of injury associated with skull fractures is abrasion (90%), followed by contused lacerated wound (76%). similar findings were observed by soni SK, et al.7 The majority of the victims in this study are of 21-30 years age group (30%), followed by 31-40 years age group. similar findings were observed by S Gowda H et al.7 and Dhakankar S et al.8

Conclusion

The conclusion of the the present study, which was carried out at CPR hospital of RCSM GMC Kolhapur, for a period of three years. This study was done on 173 cases, which were directly brought to mortuary for postmortem examination from site, irrespective of cause of injury is as follows.

From the present study we observed that, the skull fractures are most commonly seen in motor cycle accidents, followed by assault. Majority of the victims in this study are of 21-30 years i.e. nothing but the younger age group. Most common type of fracture is linear fracture, followed by depressed fracture. Most common type intracranial hemorrhage was extradural hemorrhage. The most common injury seen along with the skull fracture was abrasion.

Prevention

So road traffic accident and assault is an unfortunate economical burden for a developing country like India. Cranio-cerebral injuries due to Road traffic accidents are recognized as a major health problem causing death and disabilities among the population of this country. Mortality and morbidity due to vehicular accidents cannot be stopped completely but it can be definitely reduced by implementing strict road traffic rules as well as by improving road conditions by concerned authorities, at the same time users of the road and vehicles also realize their responsibility in following traffic rules. Awareness programmed regarding proper protective measures and effect of intoxicating substances on ones driving ability, help in preventing their injuries by accidents. Last but
not the least; better health care facilities are required for effective emergency transportation and treatment of victims of vehicular accidents.

Conflict of Interest: Nil

Source of funding: Self

Ethical Clearance: taken from EC, GMC KOLHAPUR.

References

Profile of Deaths Due to Poisoning: Autopsied at Ssims & Rc - A Cross Sectional Study

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²Assistant Professor, ³Professor & Head, Department of Forensic Medicine & Toxicology, SSIMS & RC, Davangere

Abstract

Poisoning is an important health problem in every country of the world and it is a known fact that the incidence of poisoning in India is highest and it is estimated that more than 50,000 people die every year from poisoning¹. Poisoning affects all age groups from infants to seniors, but poisoning in teens and adults are more serious and it contributes to morbidity and mortality. Due to easy accessibility of poisons and lack of awareness, self-poisoning deaths are more common. In most of the poisoning deaths, the poison consumed was unknown at the time of autopsy but Forensic Science Laboratory plays a vital role in detection of poison. With this background, the present study has been carried out to determine the profile of poisoning cases, autopsied at SSIMS&RC, DAVANGERE, KARNATAKA. The study revealed that more number of poisoning is seen in males (62.3%) as compare to females (37.7%). Common age group affected is 21 to 30 years, followed by 31 to 40 years and least in 41 to 50 years. Agriculture group is commonly affected, followed by unemployed and least in government employees. Majority of victims belongs to lower socioeconomic group (84%). The suicidal deaths by poisoning (97%) being highest followed by homicidal and accidental.

Key words: Autopsy, FSL Report and Poisoning.

Introduction

Till today poisoning remains one of the commonest causes of unnatural death. Annually it has been estimated that the health hazards are directly or in directly due to poisons is for more than 1 million illnesses worldwide, and this could be just the tip of the iceberg as most of the cases of poisoning actually go unreported and untreated, especially in developing and underdeveloped countries.¹

Poisoning being invariably medico legal in nature among fatal cases, postmortem examination is done to establish the exact cause and manner of death. Manner of death in these cases is predominately suicidal because of the general belief that it terminates life with minimal sufferings. Even accidental or homicidal cases are also reported and alleged which was more prevalent in the past as there were no well established means of detecting poison from the viscera, etc. and it was believed that if dead body was black, blue, or spotted in places or smelled bad, the cause of death was a poison. With the advent of modern techniques of chemical analysis, this method of committing homicide has lost its grounds.¹

The commonest cause of poisoning in India and other developing countries is pesticides, the reasons being agriculture based economics, poverty, unsafe practices, illiteracy, ignorance and lack of protective measures and easy availability of highly toxic compounds. ² The attempt has been made in the present study to know the commonest group or type of pesticide involved, commonest age group affected, socio-economic status, gender and occupation affected in poisoning and to know the manner of poisoning.

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Materials and Method

The present study has been carried out after obtaining the ethical clearance and consent from the relatives to take the relevant information. All the cases brought to the department of Forensic Medicine and Toxicology, SSIMS and RC, Davangere, for medico legal autopsy with history of poisoning and cases that were diagnosed as poisoning after post mortem examination during the period of one and half year, from November 2013 to March 2015. Total 61 cases were selected for this prospective study.

In all cases of poisoning the detailed history and information were collected from the police and the relatives of the deceased questionnaire, and post mortem findings were analyzed with the chemical analysis reports. In case of hospital admitted and treated cases the information’s were collected by the perusal of hospital records. In cases of allegations, information was supplemented by either visit to the scene of crime or from the photographs of the scene of crime. The cases of food poisoning, snake bite and any other insect bite envenomation and deaths due to idiosyncratic reaction to the drugs were excluded from the study group.

Meticulous autopsy was done in all cases and the routine viscera and body fluids were collected and sent to Forensic Science Laboratory for Chemical analysis and report. After obtaining the report from the Forensic Science Laboratory, the results of the report and information acquired from the relatives and the investigating officer were entered into the standard proforma for further study.

Results

Total 61 cases were selected for the present study and the following observations were made. It has been observed that maximum number of poisoning in the study population are seen in males (62.3%) as compared to females (37.7%). Out of total 61 cases males were 38 in number and females were 23 in number (Table No 1).

Table No 1: Distribution of study population according to Gender.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>23</td>
<td>37.7</td>
</tr>
<tr>
<td>Male</td>
<td>38</td>
<td>62.3</td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>100.0</td>
</tr>
</tbody>
</table>

The study revealed that more number of poisoning in the study population are seen in the age group of 21 to 30 years (34.4%), followed by 31 to 40 years (19.7%), 0-20 years (18%), above 51 years (16.4%), and the least number of cases seen in the age group of 41 to 50 years (11.5%) (Table No 2).

Table No 2: Distribution of the study population according to Age

<table>
<thead>
<tr>
<th>Age group (Years)</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-20</td>
<td>11</td>
<td>18.0</td>
</tr>
<tr>
<td>21-30</td>
<td>21</td>
<td>34.4</td>
</tr>
<tr>
<td>31-40</td>
<td>12</td>
<td>19.7</td>
</tr>
<tr>
<td>41-50</td>
<td>7</td>
<td>11.5</td>
</tr>
<tr>
<td>Above 51</td>
<td>10</td>
<td>16.4</td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>100.0</td>
</tr>
</tbody>
</table>

From the study it is observed that more number of cases seen among the agriculture group (42.6%), followed by unemployed population (32.8%) and least is seen with government employs (1.6%) (Table No 3).

Table No 3: Distribution of study population according to Occupation

<table>
<thead>
<tr>
<th>Occupation</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Government</td>
<td>1</td>
<td>1.6</td>
</tr>
<tr>
<td>Private</td>
<td>5</td>
<td>8.2</td>
</tr>
<tr>
<td>Self-employed</td>
<td>7</td>
<td>11.5</td>
</tr>
<tr>
<td>Agriculture</td>
<td>26</td>
<td>42.6</td>
</tr>
<tr>
<td>Unemployed</td>
<td>20</td>
<td>32.8</td>
</tr>
<tr>
<td>Others</td>
<td>2</td>
<td>3.3</td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>100.0</td>
</tr>
</tbody>
</table>
The study revealed that the victims belong to lower Socio-economic group are most commonly affected (84%), followed by middle class (13%) and upper class (3%) (Table No 4).

**Table-4: Distribution of the study population according to Socio Economic Status.**

<table>
<thead>
<tr>
<th>Socio Economic Status</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper</td>
<td>2</td>
<td>3.3</td>
</tr>
<tr>
<td>Middle</td>
<td>8</td>
<td>13.1</td>
</tr>
<tr>
<td>Lower</td>
<td>51</td>
<td>83.6</td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>100</td>
</tr>
</tbody>
</table>

From the study it is observed that more number of poisoning cases were suicidal (97%) in nature, followed by homicidal and accidental among the study population (Table No 5).

**Table No 5: Distribution of the study population according to manner of poisoning.**

<table>
<thead>
<tr>
<th>Manner</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accidental</td>
<td>1</td>
<td>1.6</td>
</tr>
<tr>
<td>Homicidal</td>
<td>1</td>
<td>1.6</td>
</tr>
<tr>
<td>Suicidal</td>
<td>59</td>
<td>96.7</td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>100</td>
</tr>
</tbody>
</table>

Organophosphorus (50.8%) group of compound constitutes the most prevalent type of poison involved in the study population, followed by Aluminium phosphide(26.2%), and least being Pyrethroid, Sulphuric acid, Benzodiazepine, Parphenylendiamine and Carbamate. In the present study, in 6% of cases the poisons were not detected on chemical analysis (Table No 6).

**Table No 6: Distribution of poisons detected by chemical analysis (FSL).**

<table>
<thead>
<tr>
<th>Chemical Analysis (FSL)</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organophosphorus</td>
<td>31</td>
<td>50.8</td>
</tr>
<tr>
<td>Carbamate</td>
<td>1</td>
<td>1.6</td>
</tr>
<tr>
<td>Pyrethroid</td>
<td>1</td>
<td>1.6</td>
</tr>
<tr>
<td>Parquat</td>
<td>3</td>
<td>4.9</td>
</tr>
<tr>
<td>Aluminium Phosphide</td>
<td>16</td>
<td>26.2</td>
</tr>
<tr>
<td>Alcohol</td>
<td>2</td>
<td>3.3</td>
</tr>
<tr>
<td>Sulphuric acid</td>
<td>1</td>
<td>1.6</td>
</tr>
<tr>
<td>Paraphenylendiamine</td>
<td>1</td>
<td>1.6</td>
</tr>
<tr>
<td>Benzodiazipine</td>
<td>1</td>
<td>1.6</td>
</tr>
<tr>
<td>Not Detected</td>
<td>4</td>
<td>6.6</td>
</tr>
<tr>
<td>Total</td>
<td>61</td>
<td>100</td>
</tr>
</tbody>
</table>

**Discussion**

The objectives of the present study are to ascertain the commonest gender and age group involved, occupation, socio-economic status, manner of poisoning, and the commonest type of poison involved in the study population.

Our study has showed the highest correlation with all the parameters when compared with other similar studies done in the past.

Mrinal Haloi and others had studied the cases of suspected poisoning brought to Gauhati Medical college and Hospital during the period of one year, a total sum of 96 cases of suspected poisoning deaths were analyzed. Male victims (62.50%) outnumbered Females (37.50%) and maximum number of cases was in the age group of 20-29 years. Economic status was found to be lower in 66.66% cases and maximum cases (73.95%) are from rural habitat. Organophosphorus compounds were the most common agents responsible for poisoning with 22.91% cases. Our study has shown the similar findings which were in correlation with the study mentioned above.
A study done by B.D Gupta and others for a period of one year among the poisoning cases admitted in the department of Medicine of G G Hospital, Jamnagar during the period of 1-1-2005 to 31-12-2005. A total of 268 cases were registered. A total of 124 cases were due to poisoning and 144 cases due to various bites. Of 124 cases, 89 (71.77%) were males. Majority of the victims fell in the age group of 20-29 (42.74%). Commonest poison was Organophosphorus. Majority of cases were of suicidal cause. The findings of our study were in similar and are in agreement with the above study.

A prospective study done by a Tejus Prajapati and others on poisoning cases (excluding animal bites) which were brought to the Civil Hospital Ahmadabad, from 1st October 2006 to 30th September 2007. Total 366 cases of acute poisoning were recorded over a period of one year. Of these 70.8% were males and 29.2% female. The majority (45.08%) cases were from age group of 21-30 years. 71.6% cases were from rural area. Commonest type of poison was pesticide in 33.9% cases, followed by household chemicals (26.8%), and in 74.6% of cases poisoning was suicidal. The findings of this study are in agreement with our study except the second most commonest poison, in the above study it is household chemicals, but in our study it is Aluminum phoshide, so it is in disagreement with above study.

Vishwajeet Pawar and others did a study at Mahatma Gandhi Institute of Medical Sciences, Sewagram during the period May 2007 to April 2009. During this study cases of poisoning were observed more in males than females. Male to female ratio was 1.4:1. Maximum poisoning cases were seen in the age group of 20-29 years in both sexes. Maximum poisoning cases belong to low socioeconomic group. Insecticides poisoning was the most common and among them Organophosphorus was the commonest. Incidence of suicidal poisoning cases was more than the accidental poisoning. Agriculture was the most common occupation of the victims of poisoning. These findings were in agreement with our study.

Vikram Palimar, & Prateek Rastogi did a retrospective post mortem study at Kasturba Medical College, Manipal, India, during the period of 1992-2004. Of the total 1917 autopsies conducted, 372 cases were due to poisoning, of which 287 cases were due to insecticides with a predominance of organophosphates. More than 90% of poisoning deaths were suicidal. Majority of the victims were males in their third decade of life. These findings were in consistent with our study.

A study was done by Sinha US, Kapoor AK, Agnihotri and Srivastava PC on 285 poisoning cases at SRN Hospital, Allahabad. It was see that males (69.47%) outnumbered the females. Young age group (15-30 years) was predominantly affected. Aluminium phoshide (42.1%) was the poison of choice with mortality being highest, followed by Organophosphorus (17.17%). Manner of poisoning was suicidal (87%) followed by accidental (7.37%). The findings are in agreement with the present study. But the commonest compound detected in our study is Organophosphorus (50.8%) followed by Aluminium phoshide (26.2%), so this finding is in disagreement with the above study.

In a retrospective study carried out at Department of Forensic Medicine, B.M. Patil Medical College. Bijapur, Karnataka. Total 210 fatal pesticide poisoning cases were selected for the study out of 980 medico legal autopsies conducted from 1st January 2003 to 31st December 2009. The study revealed that the age group 21-30 constitutes the majority (38.12%) of victims followed by 31-40 (25.56%). Males outnumbered the females, the male female ratio being 2.6:1. 192 (91.42%) cases were of suicidal in nature. Organophosphorus compounds were prevalent (64.3%), followed by organo-chloro compounds (25.2%) and carbamates (11.5%). The lower socio-economic status (78%) group is commonly affected. These findings were in agreement with our study except the second most prevalent poison, in the study mentioned above, Organochloro was second common compound but in our study it is Aluminium phoshide.

**Conclusion**

Following were the conclusions drawn after a detailed study of 61 cases of poisoning, autopsied at SSIMS and RC, Davangere.
1. Most of the victims are males (62.3%), where the females were of 37.3%.

2. The most common age group involved is 21 to 30 years (34.4%).

3. Most common occupation involved is Agriculture (42.6%).

4. Victims belongs to lower Socio-economic group are most commonly affected (84%).

5. Suicidal deaths were more common (96.7%) when compared to Homicidal and Accidental deaths.

6. Organophosphorus compound was the most commonly found pesticide followed by Aluminium Phosphide in the chemical analysis at Forensic Science Laboratory (50.8%).

In India, prevention of poisoning deaths poses a difficult task, as the cause of poisoning is multifaceted. The agricultural group being frequently exposed to pesticides, chances of suicide is more prevalent of various reasons like, poverty, loss of crops, unemployment and other socio-economic problem. Divorce, dowry, love affairs, illegitimate pregnancy, extra-marital affairs and such conflicts relating to the issue of marriage, play an important role, particularly in the suicide of women in India. In order to minimize the deaths due to poisoning, awareness need to be created among the public about the seriousness of poisoning, implementing the measure to uplift the socio-economic status and to solve unemployment and last but not the least, the law has to be strengthened towards the sale and distribution of pesticides. Establishing the poison control center in every treating hospital may decrease the deaths due to poisoning.

**Conflict of Interest:** Nil

**Source of Funding:** Self

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Scope of Periodontium in Forensic Science

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Abstract

Forensic odontology is the application of dental knowledge in forensic science in identification of victims during criminal justice. This article highlights the important role of gingiva and the surrounding periodontal structures in forensic field. Evidence can be collected only with proper documentation of the dental records. The importance of dental records plays a key role not only in identification but also in terms of dental malpractice or in insurance claim. Recent studies have established ante mortem and postmortem changes in gingival and periodontal tissues. Thus periodontitics has a scope in forensic odontology.

Key-words: Forensic odontology, Periodontitium, Age estimation, Dental records

Introduction

Forensic dentistry is a branch of forensic medicine dealing with evidences and marks left by teeth (as in identifying criminal suspects or the remains of a dead person). It is performed by a comparatives evaluation of the ante mortem (missing person) and postmortem (dead person).

Periodontics is a branch; which deals with clinical science of disease in periodontium. It is utilized for identifying individuals through morphology and pathology of periodontal structures and age estimation studies. Periodontal disease factors are most important for periodontics. Case documentation and photographs are proved to be the most important evidence for forensic odontology. Dentistry plays a significant role in this process. To identify the victims of crime and disaster through dental records, dentists assist those officials involved in criminal investigation. Recently, the periodontitis identified the features of ante mortem and postmortem changes in gingival tissues. In future, periodontal structures would play a crucial role in forensic odontology. This article gives an update on the assessment of periodontal structures and its uses in forensic science.

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Gingiva

Cell death occurs by apoptosis, necrosis, or autolysis.¹ Time lapsed after death is important in crime cases. Cellular change that takes place in gingival tissues helps to estimate changes after death. These changes vary in individuals at cellular level. Histological examination of gingival tissue when observed at different time intervals within 10 hours after death cellular changes occurs, in both postmortem and ante-mortem reports.² Gingival cells analysis by quantitative cytomorphometric method, involves scraping of attached gingiva to assess age and gender related alterations in the nucleus and cytoplasm. Chromatin clumping, nuclear karyopyknosis, widened intercellular junction and loss of epithelium are some of the changes at microscopic level seen at different intervals of time after death. Oral premalignant and malignant lesions, pathologic smears have cytoplasmic ratio values which showed that attached gingiva can be used for human identification.¹

COLOUR

Normal color of gingiva is pink and in some cases with pigmentation. Pigmentation is most commonly seen on labial aspect of gingiva and highest at the incisors. Pathologic pigmentations could be in various conditions like peutz jehgers syndrome, Addison’s disease, oral melanoma and melanotic macule.

CONTOUR

In healthy individuals, the gingival margin has
scalloped margin. This scalloping contour depends on location of teeth, size of proximal contact, gingival embrasures and alignment of teeth. Contour can be altered in case of improperly constructed restorations, gingival pathologies, and improper prosthetic abutments. Stillmans cleft and McCall’s festoons are changes in contour seen on canine premolar region and are usually associated with occlusal trauma.

**Position**

Gingival margin is located 1 mm above the level of cemento enamel junction (CEJ) or at the level of CEJ. Apical migration of marginal gingiva below CEJ which leads to exposure of root surface due to periodontal diseases is called gingival recession. The main etiology of recession is gingival inflammation and faulty tooth brushing. Secondary factors include anatomic, habits, iatrogenic and physiologic. Studies have proven that gingival recession varies in different races.

**Periodontal Ligament**

Morphology of periodontal ligament as age advances. Average width of ligament space is documented as 0.2mm. Considerable variation occurs in various individuals. This space is decreased around teeth that are nonfunctional and increased in hyper functional teeth.

**Thickness**

The thickness of PDL will be exactly connected to age and mesiocclusal drifting of teeth. PDL ranges in width from 0.15 to 0.38mm and progressively decreases with age. Periodontal ligament thickness is directly proportional to root dimensions.

**Widening**

Periodontal ligament widening is seen in Bisphosphate associated osteonecrosis of the jaws, progressive systemic sclerosis primary hyperparathyroidism.

**Lamina Dura And Alveolar Process**

Height, contour, density of crestal bone, tori, exospores, bone loss (horizontal/vertical) are the clinical parameters alveolar bone for establishing identity of the individuals. Height and density of crestal bone are important factors of periodontitis. Alveolar crest height loss in mandibular. The thickness and alveolar process width increase between the first premolar.

**Cementum**

Cementum is a dynamic structure that gets deposited throughout life. Cementum annulations play a role in age estimation in males and females. During the formation of cementum the alternate light and dark bands are formed from alternate deposition of hyper mineralized extracellular matrix with less mineralized layers. These light and dark bands correspond to one year of an individual. These are counted on a pictomicrograph under microscopes.

Number of Incremental lines (n) = X/Y

Where,

X=Total width of dentinocementum junction surfaces

Y=Width of two adjacent incremental lines in cementum.

Dental cementum has opaque and translucent bands in winter (dormant) and summer (growth) seasons. Dental cementum increment analysis (DCIA) is used to specify the season of death.

**Significance of Implants in Forensic Odontology**

Dental implants have a high melting point. when teeth is exposed to extreme heat, vaporization of pulp tissues occurs, leading to separation and disintegration of the tooth crown and root. Dental amalgam, composite resin and gold are the conventional restorative materials which may melt or distort in high temperature. Dental implants have a melting point above 1650 degree centigrade. Implants can sustain thermal insults which is an added advantage in identification of victims. Pre and post incineration of the laser etched batch number is also visible which depends on the depth of etching and presence of the oxidation layer on the implant surface.

Software database is also available which stores information related to implant designs, Implant systems, clinical and radiographic images which aids in crime cases.

**Dental Radiographs**

Dental radiographs are easily available and serve as a vital clue for identification. Shape of the teeth and roots, teeth present, missing teeth, residual roots, supernumerary teeth, attrition abrasion, fractures, bone
resorption due to periodontal disease, bone pathology, dental caries, endodontic treatment, intraradicular post, intracoronal post and dental prostheses treatment, are the parameters used in dental radiographs.\textsuperscript{15}

**Conclusion**

Forensic odontology having a great effect or influence in the field of dentistry, which is practiced worldwide. This article aims to enlighten the importance of forensic odontology and its relationship with periodontium. Forensic odontology plays a significant role in identifying the deceased by determining sex and age, using various techniques, including that of the gingiva and periodontium.

**Clinical implications**

This article gives an overview of structures of periodontium and its application in forensic science which implies maintaining a dental record will further aid in the process of legal issues. A dental record should contain all related information of the patient, his medical history, teeth with restorations, or pathologies, missing teeth, soft tissue changes in gingiva, alveolar bone loss, treatment planned and done and prognosis. Preoperative and postoperative photographs and radiographs all should be maintained. It would not only help in forensic odontology but also in cases of medico legal issues, insurance and consumerism. Computer generated dental records earn more benefit because it can be easily networked and for routine professional consultation forensic in cases.\textsuperscript{16}

**Source of Funding:** Nil

**Ethical Clearance:** Not required as it is review of literature

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Touch Dna as Forensic Aid: A Review

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Abstract

Touch DNA technique which is being used in many advanced countries as a modern tool in criminal justice system. It is basically meant for acquiring genetic information from biological substances (cells released from epidermal surfaces) left on touched samples to establish it’s relatedness. It refers to recovery of traces of DNA from the biological cells released during the contact which is in a very low quantity, for further analysis and generation of a DNA profile of a person. Released dead cells are not visible to naked eyes and hence it is difficult to locate and recover successfully. DNA profiling from touched samples is difficult and hence require sensitive approach in recovery, extraction and amplification. Success of Touch DNA analysis, therefore, depends upon various factors like collection, sampling and preservation, removal of contaminations, quantification, amplification, analysis and interpretations. Various methods have been developed for the collection of Touch DNA over the time. Sophisticated kits and instrument and well equipped forensic laboratories help to provide concrete DNA profiles thus helping to the Criminal Justice System.

Keywords: Touch DNA, Contact DNA, Trace DNA, DNA fingerprinting, criminal investigation, forensic science, Real Time PCR, PCR

Introduction

High profile cases including exoneration of Timothy Masters and the Jon Benet Ramsey homicide investigation has increased interest in Touch DNA1. The ability to recover DNA sample from epithelial cells was reported for the first time in 1997 by Van Oorschot and Jones. Touch DNA can be very useful in different cases, including sexual assault, rape, and murder2. Millions of skin cells are shed each day by humans and these cells may be transferred to any object coming in our skin’s contact. It is possible to deposit sufficient number of skin cells to any object at the scene of crime. The DNA from the cells of the perpetrator/ deppositor can be lifted and utilized as a potential source of physical evidence to link him with the scene of crime. DNA profiles can be obtained from the clothing that have been worn by the perpetrator act as potential physical obtained from the transferred skin cells. DNA profile from inside of the shoe can link a wearer of the shoe thus increasing the evidential value of the forensic evidence. Castella and Mangin conducted a touch DNA study on 1739 case samples and found only 26% suitable DNA profiles3. Raymond et al conducted a touch DNA study on 252 case samples and found only 44% suitable DNA profiles4.

In keeping the view of challenges various sensitive DNA typing kits have been evolved to develop DNA profiles from just a few cells having trace DNA from objects such as postage stamps, documents, bullets, knives, door handles1. Touch DNA can be recovered from a variety of items like bottles, cans, handled items, personal items, forehead marks, cutlery, food,
envelopes, letters, postage stamps, etc. Chew gums, cigarette butts, hats, upper garments, gloves, socks, shoes, underwear, etc. have been used as the potential source of the touch DNA.

This review article throws some light on the conventional to the latest sampling methods, benefits, evidential value and limitations of Touch DNA. Touch DNA Analysis requires trained laboratory personnel/investigating officers/Police personnel to lift, handle and transport exhibit from scene of crime to the forensic laboratories. Several factors affect the quality of STR profile of Touch DNA i.e. pressure, area, time and personnel’s state of mind. More the touch pressure, touch area and touch time, more is the chances of obtaining good quality STR profiles. The rough substrate has greater chances of having Touch DNA. Various methods have been developed for the collection of Touch DNA over the time. Sophisticated kits and instrument and well equipped forensic laboratories help to provide concrete DNA profiles thus helping to the Criminal Justice System.

Methods of Lifting Touch DNA Sample

Swabbing Techniques: Traditionally, dry sterile cotton wool swabs are used to lift touch DNA samples from the scene of crime called dry swabbing which are being replaced by wet sterile cotton wool swabbing. Swabbing techniques recovered less DNA, therefore, it is further advanced with tape lifting technique. Cotton wool swabs are still being used to collect Touch DNA from the handled objects. Cotton wool swabs are useful to plain substrates but results in the huge loss of DNA from the rough and porous substrate. Indianapolis implemented the use of prepackaged touch DNA swab kits (TriggerPro) for collecting touch DNA samples from a seized firearm during 2008-09. TriggerPro contained three moistened swabs with antimicrobial fluid to be used by police officers. Sterile distilled water is replaced with non-polar surfactants as wetting agents for the sterile cotton wool swabs to collect the Touch DNA from the suspected articles might be used at crime scene. As swabbing techniques recover very little amount of sample, the extracted amount of the DNA from the swab of touched area is very less.

Tape Lifting: Collection of Touch DNA via tape lifting from fabrics in many jurisdictions done routinely. Now water soluble tape lifts are available increasing the amount of touch DNA e.g. SceneSafe Fast™ minitapes.

Cutting/Scraping: The best method to obtain Touch DNA is cutting/scraping the suspected touch area but being destructive in nature generally nondestructive techniques like swabbing and tape lifting techniques are used to lift touch DNA from the scene of crime.

FTA Card: FTA Cards, MicroFLOQ swabs have been devised for direct PCR amplification reducing the chances of loss of touch DNA during DNA extraction and quantification.

Touch DNA Extraction

Bright and Petricevic conducted organic (phenol:CHCl3) and Chelex 100 resin method for the recovery of DNA on swabs collected from the hands and feet of volunteers showed that organic extraction gave increased yield. A study has shown organic DNA extraction method is most effective method for extracting trace DNA from crime scene samples in DNA laboratory. DNA extraction from saliva samples by using SceneSafe Fast™ minitapes is most efficient with conventional organic Phenol-chloroform-isooamylalcohol method on the other hand “iPrep Forensic Kit” and “PrepFiler Express BTA™ Kit” (Thermofisher) proved to be safe and fast as compared to conventional organic solvent extraction method.

Silica based magnetic automated DNA extraction systems viz. EZ1/XL BioRobot (Qiagen), Maxwell® FSC (Promega) etc.; provide a good quality genomic DNA and reduces chances of contamination during manual handling as well as rapid.

A Low DNA Content BioChipSet (LDC BCS) designed to function in the fully automated Accelerated Nuclear DNA Equipment (ANDE) performs efficient DNA purification followed by microfluidic ultrafiltration of DNA, maximizing the quantity of DNA for subsequent amplification, electrophoretic separation, and detection of amplified fragments. Recently a 96-well centrifugal filtration plate has been designed and used in an automated DNA extraction method of touched objects. 92 Samples can be processed for extraction and purification automatically on a robotic workstation in 90 minutes.

Quantification

The quantification step plays an important role in determining how a sample will be processed downstream. Advantage of DNA quantification includes
results in fewer off-scale, over & under amplified samples, gives normalized profiles, resulting in reliable data interpretation, designed to reduce the need for downstream reanalysis which enables long-term cost savings. DNA quantification can be done using various instruments i.e. UV – spectrophotometry, Micro drop / Nano drop (based on UV spectrophotometry) or Real Time PCR which provide variable data quantity of DNA from isolated samples. Real time PCR is considered above spectrophotometry as it gives actual initial concentration of human DNA being amplified whereas absorbance may vary due to contaminants.

**Amplification And Detection**

Amplification of extracted DNA is a crucial step as the whole profiling is read on the basis of those amplified loci. Multiplexing is used for STR profiling, for which various sophisticated kits are available from different manufacturers for example: AmpF/STR SGM Plus™, AmpF/STR Identifiler Plus, Globel filer™ PCR Amplification Kit system (Applied Biosystems) Investigator 24Ples QS kit (Qiagen), PowerPlex® 21/18D System (Promega) etc. by following the recommended protocols. In many cases where partial profiles are found, another approach for generating profile can be done using Mini STR kit. As initial sample concentration in such cases is too low an increase in the number of PCR cycles can result in successful STR profiling. Separation of DNA fragments is done using capillary electrophoresis by using any of the Genetic Analyzer (3100, 3130, 3500, 3500XL) and the software for data analyses is Gene Mapper ID-X software (Applied Biosystems).

**Automation in DNA Profiling**

Bio Chip Set based fully automated Accelerated Nuclear DNA Equipment (ANDE), RapidHIT ID System for Human Identification systems enables direct processing of samples right after collection. The automated process does right from cell lysis, amplification, and capillary electrophoresis to generation of STR profiling in less than 2 hours. Self-contained sample cartridges transform a multi-component protocol into a single instrument and single user-initiated task.

**Results Interpretation**

The profiles obtained from the samples are classified as either full DNA profile, partial DNA profiles, mixed DNA profiles (for profiles containing DNA from 2 or more people) or no result depending on the number of alleles detected. Touch DNA interpretation can be done after STR profiling. When two DNA profile match to each other is considered as ‘Inclusion’ and two profile not matches to each other is considered as ‘Exclusion’.

**Problems in Analysis**

**Sampling and handling problems**—Various methods i.e. swabbing, tape lifting, cutting, scraping, etc. have been utilized to obtain Touch DNA from the scene of crime. Special care should be taken while lifting Touch DNA as there are chances of DNA contamination from the person himself lifting the DNA and from the other sources at the crime scene. The method of recovering the DNA should be chosen depending on the nature of the substrate. Though swabbing techniques are simple and nondestructive, recovers less Touch DNA and have higher chances of contaminations. Only the trained-laboratory personnel/police personnel should be allowed to lift touch DNA from exhibits.

**Contamination problems**—Touch DNA analysis faces a various variety of contamination due to improper handling, sampling and preservation inhibition by bacteria or any other type of chemical such as EDTA, sodium fluoride, saline solution, microbial development and environmental effects. Contamination problems in DNA Samples increase with high demand of DNA analysis in forensic laboratories. Despite the consciousness, the risk of contamination arises from the first handler police staff, during transportation and even in forensic laboratories. Special care should be taken while lifting the touch DNA samples. High recommendations should be made for the use of aprons, face masks, head masks, gloves, sterile forceps, scissors, etc.

**Preservation and transportation problems**—Biological samples are preserved by drying using any of processes of viz. desiccation, refrigeration and freezing. Transportation of these samples requires special handling procedures without which there is chances of degradation/decomposition of biological samples rendering it unsuitable for DNA fingerprinting analysis. Delay in sample freezing and temperature fluctuation during transportation from one place to from another place makes biological samples unfit for DNA fingerprinting analysis. An emphasis is being made on the development of storage devices at room temperature, thus reducing the cost of freezing or refrigeration.
Benefits of Touch DNA

Touch DNA reflects an implicit decision that the marginal benefits of DNA collection are greater than that of additional patrol resources made available if touch DNA is not used by officers. Benefits of Touch DNA lies in its specificity. Even minute quantity of DNA from just a few cells from the scene of crime can provide complete DNA profiles of perpetrator. Touch DNA is very fast, it can be applied on a variety of surfaces, and it can be used on a very small sample. Latest more sensitive kits, sophisticated equipments have proved boon to touch DNA technology.

Limitations of Touch DNA

The amount of deposition of Touch DNA is variable due to different factors for examples on rough and porous surface as compare to smooth surfaces, and some persons are known to leave more Touch DNA while some others are known to leave less DNA, person during stress or during sweating leave more Touch DNA as compare to normal persons. Touch DNA being a biological material is prone to environmental and biological degradation by fungal bacterial contaminations.

Touch DNA samples being very small in quantity can be easily destroyed and can’t be recreated. DNA material in aerosols can contaminate the laboratory environment by making other DNA material unfit for further profiling. Touch DNA technology depends on well trained personnel. Any error during processing technique may result in incorrect or incomplete DNA profiles. False/erroneous interpretation of results can lead to wrongful conclusions which in turn mislead the case.

Conclusions

The success of Touch DNA use as a forensic tool lies in the optimization of techniques starting with collection of biological samples, preservation, extraction, amplification and genotyping. Many newer methods have been developed, sophisticated kits, instruments along with well-equipped established laboratories which will help right from recovery of biological samples at crime scene to handling to profile generation. However, a careful and meticulous planning is required success for individual cases. The limitations of Touch DNA technique should be also kept under consideration while interpreting the results.

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Flibanserin: A Miracle Drug in Management of Hypoactive Sexual Desire Disorder in Female

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Abstract

Female sensuality encircles wide range of behaviours and activities, including female sexual identity and etiquette. Many dimensions of female sexuality have been addressed by various societies and religions of the world, pertaining to biological sex, orientations and attitudes. A sufficient sexual appetite and libido in adults are essential for continuation of race. It also nurtures psychological health and immune functions. In case of hypoactive sexual desire, there will be recurrent deficiency or absence of interest and receptivity to sexual activities which causes distress and many interpersonal difficulties in fledged adult life. Flibanserin is the latest approved drug for the treatment of hypoactive sexual desire disorder in female. It generally functions on the activity and the role of neurotransmitters affecting mood and drives. Flibanserin has affinity with serotonin autoreceptor (5-HT1A) and dopamine receptor (D4). It is a full-fledged agonist of 5-HT1A in prefrontal cortex region and partial agonist in the CA3 region of hippocampus. It also acts as partial agonist of dopamine receptor (D4) but it has poor antagonist property with 5-HT2A, 5-HT2B and 5-HT2C receptors. Flibanserin declines neuronal firing rate generally in the cells of hippocampus, dorsal raphe and cortex region of brain. Hence, it can assist to improve the brain activation pattern and drives of hypoactive women.

Keywords: Flibanserin, Hypoactive women, Autoreceptor agonist, Libido, Drive disorder.

Introduction

Issues related to women’s health are widening day-by-day. According to World Health Organization (WHO), women are more prone to hormonal imbalance, while males have a great tendency to resist the change1. The physiology of female is quite dependent on her cyclic menstrual and shows severe fluctuations in case of any interference with exogenous factors such as environmental toxins, poor-diet and impaired lifestyle2. It can also be impeded during chemotheraphy, hysterectomy and after prolonged use of xenoestrogens. Xenoestrogens are the chemicals that are found in everyday usable items, such as certain food products, cosmetics and preservatives. Excessive use of those items has dreadful tendency to imbalance many hormones, such as estrogens and progesterone3.

Women’s well-being and their unique health issues are usually related to the hormonal essence in their body. Such issues include pregnancy, menopause and ambiance of female organs. Due to the sociocultural factors, discrimination among sex is also a major cause that affects women’s health and well-being. Depression, anxiety as well as hormonal misbalancing diseases are more likely to affect the women health as well as their libido4. Libido generally regarded as a person’s drive for sexual activity. It is the force that is responsible for the intimate contact between a man and a woman but if the person loses the drive then this condition may lead to hyposexuality which is also referred as hypoactive sexual desire disorder (HSDD). Many systems of the human body are involved in sexual drive such as nervous system, vascular system, endocrine system and other structures that are auxiliary in intercourse5. For the modulation of women’s sexual craving steroids are implicated such as testosterone and estradiol. Chief neurotransmitters

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responsible for the regulation of libido in humans are dopamine as well as oxytocin. Stress, illness, pregnancy and many other factors collectively affect the human sex desires. Women’s sexual problems are due to the mental and physiological reasons. In present scenario, women are gradually loosing their drives because of their disturbed life style and ill food-drink habits. Many other factors have also been seen. Libido could also be affected due to social issues, such as work and family. It could also be disturbed due to certain medical conditions and puberty. Non-sexual diseases such as diabetes, blood pressure, neurological diseases could also affect the sex desire. Hormonal alterations are the main and leading cause of loss of libido. Stunted libido can also lead to disquiets, such as dyspareunia, vaginal dryness, failure of orgasm, vaginismus and awful sex.

Material and Method

Flibanserin is a new drug approved by US FDA for the treatment of hypoactive sexual desire disorder in premenopausal women. Interpretation of the importance of the drug has been accomplished on the basis of existing literatures and researches. The concerned articles have been assessed through various electronic searches such as Pubmed, Scopus, Medline and Google scholar using different key words. Various inclusion and exclusion criteria such as age, gender and disease-stage have also been followed while assessing the literatures through the electronic databases.

Female Sex Hormone And Behaviour

The two major hormones which are responsible for the sexual activity in females are estrogen and progesterone but testosterone is also produced in small amount in women although it is a male sex hormone. Placenta also produces the estrogen amid the gestation period. When the stimuli reaches the brain and strikes the hypothalamic region it generates gonadotropin-releasing hormone (GnRH) at puberty and arouse the pituitary to produce Follicle stimulating hormone (FSH) and Luteinizing hormone (LH). FSH and LH are the two hormones indirectly responsible for the synthesis of estrogen (mainly estradiol) as well as androgens. The main hormone that is responsible for the maturation of follicles is FSH. When the level of estrogen gets elevated it stimulate the endometrial proliferation and leads to the deluge of LH. The LH rush is responsible for ovulation.

Puberty, menstruation, pregnancy as well as menopause are generally governed through estradiol. Progesterone is responsible for the preparation of uterus lining for the fertilized egg and it also supports pregnancy. Testosterone is generally produced in very small amount in females from the adrenal glands and ovaries and it is also responsible for the sexual desire, regulation of menstrual cycle as well as muscle and bone strength. An elevated levels of different sex hormones in female result in the growth of pubic and armpit hairs, breast budding, increase in stout of thighs and hips as well as maturation generally of uterus, vaginal and ovaries that may lead to the onset of menstruation. If there is hormonal imbalance in the female body it may cause certain critical conditions such as polycystic ovarian syndrome (PCOS), hot flashes and many behavioural changes. Hence, hormones do influence behaviour of women, especially desire and drives are copiously affected. The commonest categories of woman’s sexual dysfunctions are impaired desire and orgasmic dysfunction.

Sexual fragility in women

There are several factors that are responsible for women’s sexual response that includes contextual, personal psychological and biological factors. According to the American Psychiatric Association’s Diagnostic and Statistical Manual (DSM-IV-TR) women’s sexual dysfunction is usually due to the absenteeism of sexual convictions or fantasies before the sexual activity. Sexual difficulties are more prevalent in women. About 40% of premenopausal women lack engrossment in sexual drives because of certain physiological, psychotic or drug induced factors. The main focus of sexual dysfunctioning lies on the genital staging and lubrication to the fulfilment and resolution. Women’s sexual stimulation or desire is more complicated as their fantasies and emotional contact towards this feeling is complex. A woman requires increased level of emotional intimacy while stimulating for sexual adventure and amusement. Women’s sexual dysfunction may be a result of sexual response that is due to the impaired perceptions, fantasies and desires contextual, personal psychological and biological factors has led to recently published recommendations for revision of definitions of women’s sexual disorders found in the American Psychiatric Association’s Diagnostic and Statistical Manual (DSM-IV-TR). There are many factors that affect women’s sensualities and drive.
Impairment in the drive is also due to the elevated level of prolactin as it is seen in case of polycystic ovarian syndrome. The symptoms of enhanced prolactin in premenopausal women may lead to amenorrhea and galactorrhea. Prolactin generally inhibits the Gonadotropin releasing hormone. The stimulatory effect of prolactin is on the mammary cells that results in lactation. Ingurgitation of alcohol elevates the women’s sexual arousal transiently but shows negative effect after persistent use. Encephalonic arousal in the pre-frontal limbic system and amygdala elevate the feeling of love and enhances the responsiveness to ecstasy with female orgasm solely from sexual intercourse often regarded as a unique feature of human sexuality. However, orgasm from sexual intercourse occurs more reliably in men than in women, likely reflecting the different types of physical stimulation men and women require for orgasm. In men, orgasms are under strong selective pressure as orgasms are coupled with ejaculation and thus contribute to male reproductive success. By contrast, women’s orgasms in intercourse are highly variable and are under little selective pressure as they are not a reproductive necessity. The proximal mechanisms producing variability in women’s orgasms are little understood. In 1924 Marie Bonaparte proposed that a shorter distance between a woman’s clitoris and her urethral meatus (CUMD. Amygdala has dual role i.e. in sexual behaviour as well as in maternal care and extensively involved in building emotional competence, but in postpartum women amygdala is greatly responsible for maternal behaviour. Impairment in oxytocin release is also a noteworthy factor. It is an orgasmic hormone well-known by many names, such as ‘trust hormone’ or ‘lust hormone’. Oxytocin is also responsible for inhibition of fear in amygdala. Sexual dysfunction is the common issue in the perimenopausal women. The chief reason for the sexual dysfunctioning is hormonal imbalance.

Factors Responsible For Impaired Libido

Sexual drive disorders: Disorders of sex drive are basically classified in two types namely Hypoactive sexual drive disorder (HSDD) and Sexual aversion disorder (SAD). The induction of HSDD is caused by persistently deficient sexual reveries and desires for long time due to certain emotional factors, such as persistent anxiety and depression that may cause marked interpersonal difficulty also. It is also caused by certain...
physical factors like diabetes and extreme tiredness. SAD is developed as a result of persistent avoidance of all genital sexual contacts with a sexual partner due to some specific phobia.  

Effect of medicines: Decreased level of sexual desires have been seen in case of long term medications, such as anti-hypertensive medications, anti-psychotic medications, anticoagulants, monoamine-oxidase (MAO) inhibitors, sedatives and also in case of fat lowering medicines.  

Interpersonal and contextual factors: It includes the emotional intimacy of the women with her partner during sexual as well as the general activities. Deepening of emotional concerns are essential for intimate relationship. In case of fable relationship, boredom may happen between the partners.  

Psychogenic factor: Psychogenic distractions affect the sexual arousal in women. Anxiety and life-style have also indubitable impact on women’s drive. Psychological factors may include certain stress, guilt, worry, negative thoughts and memories of past.  

Depression: It is the major cause of declined sexual functioning. Change in sex drive has association with depression. A study in psychosomatic medicine revealed that females who had depression are more prone to hypoactive sexual desire disorder and about one-third of premenopausal females with hypoactive sexual desire disorder are firmly associated with depression.  

Chronic illness: Serious health issues and chronic illnesses have enormous adverse effects on biological drives. Physical and mental well-being play a significant role in libido expression and execution. Chronic diseases such as diabetes, arthritis and cardiovascular diseases have negative influence on drives and functioning.  

Hormonal factor: As women become older the levels of hormones such as estrogen, testosterone and progesterone decrepitude especially after menopause. Low levels of these hormones generally reflect negative impact on sex life. Reduced level indicates female sexual fragility and dysfunctions. Estradiol enhances the sexual desires, but testosterone is also responsible for the alterations in sexual desire of a woman. Low levels of estrogen leans the uterine lining and leads to decrease in sexual desire. Vaginal dryness, painful intercourse, mood swings and unstable sleep patterns are the symptoms of low estrogen. Therefore, hormonal imbalance is considered as a leading cause in reduction of sexual arousal and response.  

Flibanserin-A Wonder Drug  

Flibanserin is a N-alkylpiperazine compound chemically known as 1-(2-(4-(3-trifluoromethylphenyl)piperazin-1-yl)ethyl)benzimidazol(1H)-2-one as a P-Glycoprotein inhibitor. It works as a 5-hydroxytryptamine 1A (5-HT1A) agonist and 5-hydroxytryptamine 2A (5-HT2A) antagonist. Flibanserin behaves as all-inclusive agonist for 5-HT1A receptor in the frontal cortex as well as in the raphe dorsalis, however it acts as partial agonist in the subfield of hippocampus, especially in the CA3 region. Despite much affinity with serotonergic receptors, flibanserin has weak agonistic activity on dopamine receptor (D4) also. The upshots of flibanserin on intermediate biomolecule adenylyl cyclase are non-identical to that of buspirone which is also a well-known 5-HT1A receptor agonist. Flibanserin has also been found to increase the level of norepinephrine in the prefrontal cortex region (Figure 2).
Both dopamine and norepinephrine manifest significant role in sexual excitement. The norepinephrine shows exciting role in sexual arousal while dopamine has important contribution in boosting desires. Flibanserin has very weak blocking activity on 5-HT\textsubscript{2C} as well as 5-HT\textsubscript{2B} receptors. It has also been reported to decrease the level of serotonin in the prefrontal cortex with long time administrations. The receptor 5-HT\textsubscript{1A} acts primarily as an autoreceptor in brain and inhibits firing of 5-HT neurons from the nerve endings\textsuperscript{28}.

Flibanserin shows an absolute bioavailability of 33\% from oral route. It exhibits linear pharmacokinetics from dose 100mg to 250 mg in adult healthy women and achieves maximum concentration after 45 minutes of administration. Flibanserin shows high protein binding property and extensively undergoes first-pass metabolism through hepatic isoenzymes. The endorsed dose of flibanserin is 100 mg at bed time and maximum course of medication is up to eight weeks\textsuperscript{29}. The drug is now defined as a key drug in the treatment of female sexual interest / arousal disorder (FSAID) in premenopausal women.

**Conclusion**

Flibanserin is a new drug used to treat hypoactive sexual desire disorder in female and has wide link with 5-HT\textsubscript{1A} autoreceptor. It has also antagonistic relations with 5HT\textsubscript{2A}, but preferentially stimulates 5HT\textsubscript{1A} receptor and has also been found to increase the levels of dopamine and norepinephrine in the prefrontal cortex. It is generally used in the treatment of premenopausal women. Flibanserin is the first US-FDA approved drug for stimulating the female sexual desire. The drug is benzimidazole compound and generally enhances the sexual performance as well as number of satisfactory events of a month in premenopausal women. Flibanserin is available as 100mg tablet which should be taken orally once at bedtime. Bioavailability of Flibanserin is 33\% and 98\% of the drug shows high bound to protein mostly albumin while the half-life is of 11 hours and this drug is generally metabolized by liver enzymes. The woman who receives flibanserin experiences significant increase as compared to placebo in the frequency of sexual events. Female sexual disorder is also known by a term ‘female sexual interest/arousal disorders (FSIAD)’. It includes the changes in orgasm, reduced genital and non-genital sensations. Low libido is mostly recorded sexual problem with pervasiveness of 38.7\% as compared to 10\% prevalence when patient feels low desire along with distress. Many agents have been discovered to treat the patient suffering from female sexual desire generally low libido. But Flibanserin is the first FDA approved drug for the treatment of low libido.

**Figure 2: Flibanserin - mechanism of action**
or impaired sexual fantasies.

**Ethical Clearance:** This article has been routed through the anti-plagiarism cell of Institutional Review Board.

**Conflict of Interest:** The authors declare that they have no conflict of interests.

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**References**


Canine Width as a Means for Stature & Sex Prediction

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Abstract

Background: Estimation of stature, along with sex, age and race, is one of the four pillars of forensic anthropology and is considered a preliminary screening in identification of skeletal remains. Teeth, particularly the canines form an excellent material for such forensic investigations as they resist decay due to caries or other diseases of tooth as well as remain highly stable during natural calamities.

Materials & Method: Study was conducted in SSSMC, Dehradun among 100 healthy subjects (>17 years), comprising 66 males and 34 females. Mean mesio-distal width of mandibular canine tooth was measured on both side on dental casts and mean stature measured by anthropometer. The collected data was subjected to statistical analysis; correlation and sexual dimorphism was calculated for both sexes and a linear regression formula obtained for estimating stature.

Observations & Result: Mean canine width on both right and left side as well as height was more in males as compared to females. Sexual dimorphism was more involving right canine width. The findings were found to be statistically significant for right canine width (RCW) and for height, between male and females. Accuracy for sex prediction was high in females (F= 64.7%, M=59.1%). Using the values of RCW and LCW, regression equations were obtained for estimating height.

Conclusion: Width of mandibular canine is reliable for stature estimation but for sex prediction it is not much reliable on the left side. However these parameters can be used as an adjuvant in situations when only single tooth is available for identification.

Keywords: canine width, stature, identification, sexual dimorphism

Introduction

In mass disasters, identification of an unknown person from fragmentary remains is the utmost requirement in order to aid the legal authorities in further investigation. Significant parameters for establishing the identity include age, sex and stature because as only these criteria needs to be considered and rest all can be excluded in the missing persons¹. A definite biologic relationship exists proportionately with every part of the human body including head, face, trunk, long bones, foot length as well as shoe print.² ³ Forensic dentistry too plays an important role in human identification, especially when conventional methods cannot be applied, usually due to advanced decomposition, carbonization or fragmentation of the body.⁴ ⁵

The method of using teeth has several advantages as it is easy to locate & measure. Also, they are relatively resistant to damage and most of the odonto-metric parameters remain constant over time⁶ ⁷. Amongst all the teeth in human dentition, canines are the most stable because of the labio-lingual thickness of the crown and root anchorage in the alveolar process of jaws. They are the least frequently extracted tooth or affected by periodontal diseases, less exposed to plaque, calculus and abrasion from brushing. In fact, they are

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reported to withstand extreme conditions and have been recovered from human remains even after hurricanes and air disasters\textsuperscript{13-16} Though, most of studies have been conducted to correlate combined mesio-distal width of maxillary or mandibular anterior teeth with stature and sex prediction due using various indices; this study aims to study the relationship of only mandibular canine with stature and sex which could prove to be useful when disarticulated tooth from the socket is recovered in above mentioned situations.

**Materials & Method**

The study was conducted in Shridev Suman Subharti Medical College, Dehradun among 100 (66 males & 34 females) healthy students of the Institute having age > 17 years. Those having broken/ damaged or caries involving canine, spinal deformity (kyphosis, scoliosis etc.) or any history of orthodontic/ orthognathic treatment were excluded from the study. A written informed consent was duly obtained from all the willing participants; procedure and purpose of study was explained in detail. Stature was measured as the vertical distance from the vertex to the floor using a standard anthropometer. Measurements were taken by making the barefooted subject stand erect on a horizontal resting plane. Anthropometer was placed in straight vertical position behind the subject with the head oriented in the Frankfurt Horizontal Plane & shoulders & hips touching the vertical limb of the instrument. The movable rod of the anthropometer was brought in contact with the vertex in the mid-sagittal plane. A dental cast of lower jaw was prepared for all the subjects using alginate paste and dental stone. Maximum mesio-distal width of mandibular canines was measured directly on these casts using a digital vernier calliper. It was noted by a single observer on both sides to minimise subjective errors; all these measurements were taken twice and an average was duly noted. Subsequently, using statistical software SPSS version 20, sexual dimorphism, Pearson’s correlation and simple linear regression equations formulated for determining stature.

**Observation & Results**

Among the subjects in the study, mean canine width on both right and left side was more in males as compared to females. Similarly, height was found to be more in males. In all the parameters, t value expressed a very slight difference between male and females, in a positive direction. These findings were found to be statistically significant for right canine width and for height between male and females since p value (0.032) is less than 0.05. However, they were found to be insignificant for left canine width. Sexual dimorphism is highest when height is being considered, but among canine width it is more on the right side. (Table 1)

<table>
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<th>Table 1: Independent sample t-test</th>
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NOTE: RCW-Right canine width, LCW-Left canine width, H-Height, N-Number, SD-Standard deviation

Paired t test showed that in females LCW > RCW as the t value obtained is –0. 596 while in males RCW > LCW as t value is 0.170. However, there is statistically non-significant difference between right and left canine width in both the sexes as p value is more than 0.05. (Table 2)
The percentage of accuracy in predicting sex was found to be 64.7% for females and 59.1% for males by using Discriminant function analysis.

In females, the Pearson’s correlation value between height and canine width on both sides is found to be positive and very weak having statistically non-significant correlation as p value is more than 0.05. Similarly, in males, correlation value between height and RCW is positive and very weak; statistically non-significant correlation. However, on left side (LCW) there is statistically significant correlation. Also, in total subjects, correlation value is positive, very weak and found to be statistically significant. Using the values of RCW and LCW as independent variable, regression equations were obtained for predicting height in both the

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<th>Table 3: Simple linear regression equations (height as dependent variable and canine width as independent variable)</th>
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Discussion

Since no two dentitions are alike, this dental identification can be applied for differentiating the size, stature and appearance between male and females. Various studies have been conducted in the past to predict sex from tooth measurements and to determine sexual dimorphism. It has been found that highest sexual dimorphism is observed with canines as compared with other teeth.

Most of the authors failed to obtain a significant difference in canine widths between the right and left sides, which is consistent with our study. Also, statistically significant differences were obtained by some authors in mesiodistal widths of mandibular canines between males and females, which is inconsistent with our study. This significant sexual dimorphism in the tooth can be attributed to the presence of relatively more dentine in males when compared with females.

Similar to our study, some of the authors found a higher sexual dimorphism in right canines as compared to the left canines except in the study by Kaushal et al who found a higher sexual dimorphism on the left side.

Numerous studies have been conducted considering various odontometric parameters either individually or collectively, having some degrees of positive correlations with stature with or without significance between them.

Yadav AB et al conducted a study involving individual tooth dimensions of maxillary canines which revealed that that all odontometric parameters showed positive correlation with stature independent of gender; among them maxillary canine width showed highest correlation. Few studies involving only crown dimensions of teeth failed to provide accuracy of estimation of stature due to significant but low to moderate correlations. This is in concordance with our study to some extent as we did observe statistically significant correlation when all the subjects were considered together and among the male on left side. These relatively non-significant findings could possibly be attributed to early completion of growth of tooth crowns as compared to other parameters such as long bones which on the other hand mature much later and have a higher stature-correlation.

Conclusion

From the present study, it can be concluded that regression equations generated from meso-distal width of mandibular canine can be used as a supplementary
approach for predicting stature when limbs/extremities are not available or are fragmented. Since, there is statistically significant difference between male and female only for RCW and sexual dimorphism is more on the right side, sex prediction is not much reliable using measurement of left canine. Also, accuracy for sex prediction using canine width as a parameter is more in females (64.7%). However, these parameters are population specific and anthropometric difference varies between races. It is also invariably influenced by national, social and economic conditions. Hence, the findings need to be used with caution and the study requires to be extended further to involve different population groups.

Conflict of Interest: None

Funding: Self

Ethical Clearance: obtained from the Institutional ethics committee

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Introducing Micro-hardness Test in Forensic Odontology as an Aid in Solving Crimes: Multidisciplinary Approach

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Abstract

Background: Many studies have used dental radiography, light microscopy and electron microscopy to study the incinerated tooth for a possible estimation of temperature. However, no study so far has focused on measuring the change in hardness of dental hard tissue owing to higher temperatures.

Objective: To introduce a novel method which may indicate the approximate temperature to which dental hard tissues (teeth) might have been exposed.

Methods: We utilized Vickers hardness testing machine on a set of unrestored, non-carious extracted human teeth which were grouped and exposed to particular temperature of 200, 400, 600 and 800 degree Celsius respectively. Vickers Hardness Number (VHN) was obtained by measuring the diagonals formed after indentation at specific weight and time (constant) for all specimens in every group. Optical Photomicrography was used to view the enhanced images of indentation. The value was computed for all specimens in every group.

Results: We observed overall reduction in VHN values with increase in specified temperature. VHN of enamel was limited to 200 and 400 degrees Celsius, whereas VHN for dentin greatly reduced at 600 and 800 degree Celsius by 10 times. Surprisingly, VHN values for enamel were higher for incinerated tooth (at 200 and 400 degrees Celsius) than VHN reported in literature for non-incinerated tooth.

Conclusion: We suggest that micro-hardness test in forensic odontology is feasible and can be added in the list of already existing techniques for temperature estimation however, further experiments are recommended for its reproducibility.

Keywords: Forensic dentistry, Forensic Science, Hardness test, high temperature, dentin, incinerated tooth, Microscopy, Dentition, Body remains, Enamel

Introduction

Dental structures are the hardest and well protected structures in the body. These structures resist decomposition and high temperatures and are among the last ones to disintegrate after death.1 Incinerated human remains often require dental comparison to establish ones’ identity. These remains are often fragile and vulnerable to damage teeth and facial bones on minor forces, disrupting anatomical relationships, and impairing the ability to compare with ante mortem records.2

A systematic, conservative approach prevents the loss of valuable dental information before a thorough picture of the individual’s dental remains has been
adequately documented. The presenting conditions of fire victims are often explained and illustrated with photographs, with a series of illustrations and text describing the damage seen in the dentition of the fire victims. Studying the effect of heat on teeth and the restorations would help ascertain the temperature reached by the fire, which would be highly crucial in cases where skeletal remains are recovered. This general estimation of burning temperature could provide important information to reconstruct a criminal act.

Various studies have been done to examine physical changes such as change in colour, texture, or morphology that occurs after exposure of teeth to high temperatures in an incremental pattern. Microscopic, radiographic and ultra-structural examination using scanning electron microscope have also been documented. In the present study we propose a novel method which may provide reliable range of temperature at which a dental hard tissues (teeth) could have been exposed.

**Materials and Method**

Permission to conduct the study was obtained from concerned authorities of the institute. Since the present study did not involve any human participants, the procedure followed were in accordance with the standards from Declaration of Helsinki and its amendments. Sixteen unrestored, non-carious extracted human teeth stored in 10% formalin were conveniently collected and categorized into 4 groups [Group A, Group B, Group C and Group D]. Each group consisted of one central/lateral incisor, one canine, one premolar and one molar [no distinction was made with respect to maxillary or mandibular teeth and the sequence of teeth]. All the teeth specimen were then removed of any soft tissue using a hand surface scaler.

The teeth specimen were then vertically mounted on a tray that could withstand high temperature (Figure 1) and each group was subjected to particular temperature of 200 C, 400 C, 600 C and 800 C [C – Degree Celsius] respectively in an electric muffle furnace (figure 2). The increase in temperature was gradual, starting at 18 C, with an increment of 26 C/min, till the desired temperature was obtained. The teeth specimen were then carefully placed inside the muffle furnace for 15 minutes and then carefully removed at the same temperature. This ensured that the same temperature was maintained when the specimen were removed. The teeth specimen were not observed for any morphological changes.

These specimen were then semi/half-embedded in self-cure resin blocks of uniform dimensions. The specimen were embedded in such a way that the labial surface of the crown and root were almost parallel to the upper surface of the resin block. Each block had teeth specimen which were exposed to particular temperature. These blocks were labelled as Block 1, Block 2, Block 3 and Block 4. Due care was taken to minimize the damage during handling of the specimens.

The resin blocks were filed to reduce the thickness if required to permit ease of procedure. The teeth specimen was not filed to prevent any intentional damage. The embedded teeth specimen were then subjected to micro hardness testing using a micro hardness tester (Shimadzu HJV – G20ST) [figure 3] with square based diamond indenter of 1360 with a constant load of 500 gm for 15 seconds. The minimum spacing between indents was 40µm. Each test condition with the same load and time was conducted on every specimen of the block. Indents having well-formed sharp edges were considered for every specimen in all resin blocks. A total of three reading per specimen was included in calculation. This was followed by calculating average Vickers Hardness Number (VHN) for each resin block irrespective of individual specimen. The VHN values for every indent was automatically calculated and displayed on the screen installed on the micro hardness testing machine. The accuracy of the indentation was determined using optical photomicroscope with 100X resolution. [Figure 4] The procedure was limited to coronal part only since, in-vivo the radicular part of tooth is embedded within the alveolar bone.

**Results**

The VHN for each group exposed to a particular temperature is shown in table 1. The VHN for Group C and Group D was calculated for dentin. The VHN for un-supported enamel could not be estimated. A decrease in VHN was observed from group A to group B. The VHN for dentin also decreased from group C to group D. The magnified images of indentation for each group (specific temperature) is shown in figure 5. The VHN number could not be obtained for few teeth specimens since the optical microscope could not establish accurate indentations.
Table 1: VHN values obtained across different groups for enamel and dentin

<table>
<thead>
<tr>
<th>GROUP A</th>
<th>GROUP B</th>
<th>GROUP C</th>
<th>GROUP D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean VHN (Enamel)</td>
<td>448.5 ± 16.8</td>
<td>406.8 ± 41.3</td>
<td>Indentation could not be performed on enamel, rather done on Dentin*</td>
</tr>
<tr>
<td>Mean VHN (Dentin)</td>
<td>Indentation done on Enamel#</td>
<td>42.5 ± 2.1</td>
<td>39.7 ± 1.6</td>
</tr>
</tbody>
</table>

*Indentation not possible since Enamel was chipped off from DEJ at high Temperature

# Enamel was intact at lower temperatures; Indentation was possible. VHN - Vickers Hardness Number

Figure 1: Tooth specimens mounted on tray [to withstand high temperatures]

Figure 2: Electric Muffle furnace used in the study to incinerate tooth specimens.

Figure 3: Micro-hardness tester [Shimadzu HMV-G20ST, v1.03]
Figure 4: Optical photomicroscope used in the present study

Figure 5: Indentations at different level of temperatures viewed using optical microscope

[Indentation diagonals measured as shown in figure 4(d)]
Discussion

There is no standard condition for enamel and/or dentin micro-hardness testing for normal teeth and perhaps neither for incinerated teeth. In the present study, we propose micro-hardness test as a novel method for incinerated dental hard tissues (teeth) for estimation of higher temperatures. Given the paucity of literature on the concept that was experimented in the present study, we took the liberty to select the testing conditions of incinerated dental hard tissue (teeth).

There is literature to support that fragmentation is an important complication when human remains are exposed to higher temperature. This also decreases the hardness of teeth structure and makes it more brittle. We have made an attempt to overcome the above mentioned characteristic using a multidisciplinary approach. To our knowledge, there is no other study reported in literature which have implemented the concept of micro-hardness testing for estimating temperatures in forensic odontology.

Micro-hardness tests are commonly used to study the physical properties of materials, and they are widely used to measure the hardness of teeth. In the present study, we chose Vickers Hardness over Knoop Hardness since, the test indentation is very small in Vickers and hence it is more useful in testing very thin materials, surface of a part or small areas. Gutiérrez-Salazar and Reyes-Gasga also proposed that in tooth hardness studies the Vickers indenter is more useful than the Knoop because a square shape indent is always conserved, and because the indentation produced on a non-flat surface, or by the difference in hardness of enamel and dentin, is easily detected.

In the present study, it was interesting to find that VHN values for enamel were slightly higher at 200 C and 400 C respectively than the VHN values of non-incinerated teeth reported in literature. Lupi-Pegurier et al reported micro-hardness of enamel in the range of 322 to 353 VHN, Chuenarrom et al reported in the range of 316 to 328.4 kg/mm² with the exception of 418 ± 60 VHN reported by Colly et al. We would also like to highlight that the above VHN values mentioned are irrespective of different loads and time duration. In the present study we set our load at 500gm for 15 seconds and the corresponding mean VHN values obtained were slightly higher. The underlying reasons for this interesting observation needs to be further explored. VHN values for enamel at higher temperatures could not be determined since at temperature above 600 C enamel if present was very brittle and limited to few surfaces and at 800 C enamel got chipped off from DEJ and its remnants were in small pieces which often go unnoticed in fire debris. However, these unsupported enamel were also tested for micro hardness testing but could not sustain the force of indenter at 500 gm. Such unsupported enamel needs to be further tested at lower loads and time.

VHN values for dentin were limited to 600 C and 800 C, since enamel was either present in bits and pieces or completely fragmented thereby exposing the dentin. VHN values could not be obtained at 200 and 400 C. This is because we had no baseline values for dentin prior to incineration, which requires slicing of specimens at the beginning of experiment (which we did not) prior to incineration. Hence VHN values obtained after slicing the specimens post incineration would not have helped us to a large extent. However, this shortcoming needs to be rectified in further experiments for more detailed information. We have compared the values with findings previously reported in literature and found that VHN values for dentin, post incineration were slightly lower than for non-incinerated dentin. This difference in temperature and its associated reduction in VHN values at higher temperatures might prove beneficial in forensic dental sciences.

It is evident from the present study that micro-hardness tests can be applied to estimate the temperature range to which the dental hard tissues (teeth) might have been exposed. These temperature ranges vary according to site involved, the oxidant involved, duration of exposure to fire and burning atmosphere. For example, cremation occurs at temperatures ranging from 871 C to 982 C and combustion of petrol occurs at a temperature of 800 – 1100 C. With increase in temperature we believe the values of micro-hardness tests will also vary. Another added point is that the values are provided as an automated output from the micro-hardness tester which eliminates the possibility of any subjective bias. It also provides a feasible method to store teeth specimens/samples (embedded in resin blocks) for further evaluation if required. The values in the present study provides average VHN for a group of teeth exposed to particular temperatures. We have not made any attempt to provide individual VHN values for incisors, canines, premolars and molars. This was not included due to insufficient time and funds. Further studies are recommended to overcome the shortcomings
of the present study.

With not much literature to help us with the concept, we highlight the limitations of this experiment. They are a) Small sample size b) The VHN values were not recorded before the samples were exposed to higher temperatures: Had we recorded the values, it would have helped us to statistically analyze the results for more scientific validity. However, we have referred to VHN values from the literature to compare the findings. c) The VHN was not determined for dentin at lower temperatures: This would have involved slicing the mounted samples. Teeth become brittle at higher temperatures, the slicing was avoided since we weren’t sure if the resultant samples would be ideal for micro-hardness testing at load as high as 500 gm which was used in the present study. d) The VHN values are obtained only from the indentations on buccal surface of the samples: There are variations in the thickness of enamel on occlusion, middle third and cervical third near the Cemento Enamel Junction (CEJ) on buccal and lingual/palatal surfaces. Any information of VHN at various anatomical regions of enamel (tooth) will improve its sensitivity. e) The load and time duration was constant in the present study; though there is no set value [load and time] for either dentin and/or enamel, variation in time and load across different areas of incinerated teeth might provide more reliable results.

**Conclusion**

The present study provides a rough outline to map the changes associated with higher temperatures using micro-hardness test. It can be concluded from the present study that VHN values of enamel slightly increase than the normal at 200 C and 400 C, whereas, VHN values for dentin decrease at higher temperatures of 600 C and 800 C, for a set load and time duration. We again reinforce that further experiments are recommended to obtain more reliable results which may aid in providing estimated temperatures to which dental hard tissue might have been exposed.

**Source of Funding:** None

**Conflict of Interest:** None

**Statement of Human Rights:** Mentioned

**Acknowledgement:** We acknowledge the support from Department of Metallurgy and Materials Engineering, National Institute of Technology, Surathkal, Karnataka, (NIT-K), India for providing Vickers Hardness Number (VHN) values using the micro hardness tester (Shimadzu HMV – G20ST, v1.03)® and Optical Microscope to photograph the magnified indentation.

**References**

11. Chuenarrom C, Benjakul P, Daosodsai P. Effect of


Effect of Plyometric Training on Vertical and Horizontal Jump in Recreational Athletes Indulging in Jumping Activities

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Abstract

Background: Plyometric training is effective to improve the performance of the athletes by increasing the speed power and ability to do the activity. Most of the recreational athletes have no idea about the proper training in the field and they are very prone to get injury. This study was designed to provide the proper training and prevent further injuries in the athletes.

Objectives: To find out the effect of plyometric training on vertical jump height and horizontal jump height in recreational athletes indulging in jumping activities according to age, gender, body mass index and years of playing.

Methodology: 61 subjects participated in this study according inclusion and exclusion criteria. Prior consent was taken. Pre and post assessment of the vertical and horizontal jump height was taken. The protocol was for 3 days per week for 6 weeks. Later evaluation and interpretation of data was done.

Result: There is significant comparison between the pre and post treatment ($p<0.0001$) of horizontal and vertical jump height. Even there is positive correlation of years of playing in week 6 ($p=0.58$) for horizontal jump height and in week 6 ($p=0.43$) for vertical jump height. There is negative correlation of age in week 6 ($p=0.002$) of horizontal jump height in week 6 ($p=0.31$) of vertical jump height.

Conclusion: This study concluded that the effect of plyometric training was seen in recreational athletes indulging in jumping activities.

Keywords: Athletes, plyometric training, vertical jump height, horizontal jump height.

Introduction

Athletes involved in jumping activities are greater risk for sustaining non-contact injuries when compared with other counter-parts[1]. During the maximum running speed phase, forward propulsion of the body is determined mainly by the action of the hip extensors and ankle extensors[2]. There are various type of jumps that athletes do during their practices which can use the muscle power of the leg mostly, so the clinician use better prognostic or diagnostic value[3].

Recently, weighted-vest jumping has shown to enhance jump performance in athletic populations[4]. Recent findings from training studies of kinematic and kinetic data suggest that lower extremity malalignment is related to inefficient neuromuscular control strategies[1].

The application of the strength and power occur under condition by the type of posture, contraction type and movement pattern during the activity[3].

Vertical jump is the act of raising one’s centre of gravity higher in the vertical plane solely with the use of one’s own muscle[5]. It is divided into two types:

a) Standing vertical jump: It refers to vertical jump done from a stand still with no steps involved.

b) Running vertical jump: It refers to vertical jump after an approach or run to help add energy to the jump in an effort to improve on the standing vertical jump[5].

Sometimes seem that horizontal assessment, which involved both vertical and horizontal propulsive forces
which predict those activities that involve horizontal linear motion. In training the players, there are many factors which can be used to improve their performances in the challenging phase. The most challenging for them is the physical fitness and capacities of the player to adapt the exercises. Thus it is important to evaluate the current training and practices to provide sport-specific temporal and kinetic parameters.

Plyometric exercises are defined as eccentric loading immediately followed by a concentric contraction. Plyometric exercises involved stretching the muscle immediately before making a rapid concentric contraction. The combined action is called as stretch-shortening cycle. The rapid eccentric loading phase is called stretch phase and concentric phase is called shortening phase and the period between the stretch and shortening is called amortization phase. During this phase the muscle reverses its action, switching from deceleration to acceleration of the load. In power-related sports, plyometric training is an important component of athletic preparation to increase muscle power.

Plyometric training is thought to utilize the series-elastic properties of connective tissues and the stretch reflex of the neuromuscular unit. During the initial stretch phase the muscle contracts eccentrically and lengthens under tension. The eccentric contraction is thought to prepare the contractile elements of the muscle for the concentric contraction by stimulation and activation of the monosynaptic stretch reflex. Then the receptors send the information to the central nervous system through afferent pathways. From the efferent pathways the signal return and the reflex facilitated the activation of the shortening contraction.

Plyometric training increases performances and decreases the risk of injury in players. There are very rare studies done on the horizontal jump in player indulging in jumping activities. Tobin and Delahunt said that plyometric exercises were able to elicit a potential response in professional rugby players. Complex training is considered by strength and conditioning coaches to be a superior way to develop athletic strength, speed, and power. Plyometric should not be given in presence of inflammation, pain or significant joint instability.

Plyometric training has been proposed for the development of explosive-power performance and specifically for the improvement of vertical jump ability. As the lower extremity is subjected to high joint loads and velocities during plyometric activities, so these exercises are ideal for encouraging the reflexive pathways of feedback motor control. Plyometric training is an established technique for enhancing athletic performance but may also facilitate beneficial adaptation in sensorimotor system that enhance dynamic restraint mechanisms and correct faulty jumping mechanisms. Researches have suggested that plyometric exercises were initially utilized to enhanced sports performance and more recently used in the rehabilitation of the injured athletes to help in participation for a return to sports.

Researches have investigated the relationship between leg power and functional performance have mostly used bilateral vertical jump assessment to predict the activities. Basically there are various component are used to assess the body fat in context to the physical fitness, which are commonly used to determine health related physical fitness.

Wilt suggested that muscular performance gains after plyometric training are attributed to these neural adaptation and it may enhances neuromuscular function. Plyometric training program had positive effect in improving power and agility, and players use this training method during competitive phase.

Gambetta even suggested that using plyometric exercise in athletics training is essential, as it has become an important part in physical preparation programs utilized to developed leg explosive power especially for long and high jump which require the combination of speed and strength. In general, plyometric training is commonly thought to be more effective training exercise than training including countermovement jumps due to an increased stress for the lower leg muscle.

Loturco et al. compared the effect of unloaded vertical vs. horizontal plyometrics on sprint performance in soccer players and found out that horizontal jump group gave greater improvement in speed capacity over short duration, where as vertical jump showed superior improvement over long distances. Luebbers et al. showed that short-term plyometric training result in significant in vertical jump performance in physically active college-aged men.

Rosas et al. analyzed the effect of jumping with or without haltere type handheld loading on vertical and horizontal jump performance in youth soccer player and
the found out that they improved jump performance after 6 week, however loaded jump group showed greater improvement in jump capacities\cite{20}.

**Methodology**

61 subject were taken for the study according to inclusion and exclusion criteria. Inclusion criteria were age group was between 15-30 years and recreational athletes indulging in jumping activities like hurdle jump, long jump. Exclusion criteria were Players have previous injuries in lower extremity or have gone through previous surgeries or having ankle instability. Prior consent was taken. Pre and post assessment of vertical and horizontal jump height was taken. The protocol was for 3 days per week for 6 weeks. Each session was of 60 min.

**Exercise protocol** was started with warm-up exercise that is jogging, stretching, shoulder exercise, skipping. Then Landing drills, take off drills,full speed 30-50 meter, squat jumps, depth jumps, box jumps, lateral jumps, ankle hops, double leg jump forward and backward, double leg zig-zag hops, cone jumps. Cool down period was 15 minute.

**Statistical Analysis**

Statistical analysis of the recorded data was done by using the software SPSS version 2.0. mean+standard deviation and paired t-test were used for analysis of data. Correlation of the result with age, body mass index and years of playing were determined by using Spearmen’s correlation coefficient.

**Findings**

**Table 1: Comparison of horizontal between pre and post for week1 and week6**

<table>
<thead>
<tr>
<th>Horizontal</th>
<th>Pre</th>
<th>Post</th>
<th>Mean difference</th>
<th>95% CI</th>
<th>Z-value</th>
<th>p-value</th>
<th>Interference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week1</td>
<td>208.75 ± 21.21</td>
<td>211.60 ± 21.19</td>
<td>2.84</td>
<td>2.73 – 2.96</td>
<td>6.93</td>
<td>&lt;0.0001</td>
<td>Significant</td>
</tr>
<tr>
<td>Week6</td>
<td>226.93 ± 21.3</td>
<td>230.2 ± 21.5</td>
<td>3.28</td>
<td>3.14 – 3.42</td>
<td>6.85</td>
<td>&lt;0.0001</td>
<td>Significant</td>
</tr>
</tbody>
</table>

**Interpretation:** In the present study pre interventional mean and standard deviation of horizontal jump height of week 1 is 208.75±21.21 and of week 6 is 226.93±21.3 whereas post interventional mean and standard deviation was 211.60±21.19 of week 1 and 230.2±21.5 of week 6. It concluded that p-value was <0.0001 and interference was considered significantly. This was calculated by paired t-test.

**Table 2 : Comparison of vertical between pre and post for week1 and week6**

<table>
<thead>
<tr>
<th>Vertical</th>
<th>Pre</th>
<th>Post</th>
<th>Mean difference</th>
<th>95% CI</th>
<th>Z-value</th>
<th>p-value</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week1</td>
<td>28.14 ± 4.31</td>
<td>30.89 ± 4.23</td>
<td>2.75</td>
<td>2.67 – 2.83</td>
<td>6.97</td>
<td>&lt;0.0001</td>
<td>Significant</td>
</tr>
<tr>
<td>Week6</td>
<td>46.16 ± 4.31</td>
<td>49.29 ± 4.42</td>
<td>3.14</td>
<td>3.0 – 3.28</td>
<td>6.86</td>
<td>&lt;0.0001</td>
<td>Significant</td>
</tr>
</tbody>
</table>

**Interpretation:** in the present study pre interventional mean and standard deviation of vertical jump height of week 1 is 28.14±4.31 and of week 6 is 46.16±4.31 whereas post interventional mean and standard deviation was 30.89±4.23 of week 1 and 49.29±4.42 of week 6. It concluded that p-value was <0.0001 and interference was considered significantly. This was calculated by paired t-test.
### TABLE 3: Correlation of year of playing with horizontal jump height

<table>
<thead>
<tr>
<th>Year of playing</th>
<th>Z-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week1 – post</td>
<td>0.16</td>
<td>0.23</td>
</tr>
<tr>
<td>Week6 – post</td>
<td>0.26</td>
<td>0.05</td>
</tr>
</tbody>
</table>

**INTERPRETATION:** In the present study the correlation of the years of playing with horizontal jump height is positive by using paired test. The finding showed that years of playing in relation with the players is equally effective.

### TABLE 4: Correlation of year of playing with vertical jump height

<table>
<thead>
<tr>
<th>Year of playing</th>
<th>Z-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week1 – post</td>
<td>0.024</td>
<td>0.86</td>
</tr>
<tr>
<td>Week6 – post</td>
<td>0.104</td>
<td>0.43</td>
</tr>
</tbody>
</table>

**INTERPRETATION:** In the present study the correlation of years of playing with vertical jump height is positive using paired test. The finding showed that years of playing in relation with the players is equally effective.

### TABLE 5: Age correlation with vertical jump height

<table>
<thead>
<tr>
<th>Age</th>
<th>Z-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1- post</td>
<td>-0.234</td>
<td>0.069</td>
</tr>
<tr>
<td>Week 6- post</td>
<td>-0.312</td>
<td>0.015</td>
</tr>
</tbody>
</table>

**INTERPRETATION:** In the present study the correlation of age with vertical jump height is negative.

### TABLE 6: Age correlation with horizontal jump height

<table>
<thead>
<tr>
<th>Age</th>
<th>Z-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1- post</td>
<td>-0.128</td>
<td>0.326</td>
</tr>
<tr>
<td>Week 6- post</td>
<td>-0.392</td>
<td>0.002</td>
</tr>
</tbody>
</table>

**INTERPRETATION:** In the present study the correlation of age with horizontal jump height is negative.

---

**Discussion**

Vertical jump height and horizontal jump height are used to measure the athletes performances\(^5\). The distance achieved during the jump are dependent on the athletes ability to transfer the linear momentum force directly from the ground to athletes body’s center of mass which is critical to break the inertia and attain high velocities over short-distances. Plyometric training is helpful in increases performances and decreases injury in players\(^1\). During the maximum running speed phase, forward propulsion of the body is determined mainly by the action of the hip extensors and ankle extensors\(^2\).

The jump height is recorded as distance score. It can be affected by the angle of knee bending, effective use of hands, coordination etc.\(^5\). To achieve the maximum height during jump the athletes center of mass need to be as high as possible in relation to the ground to attain the highest velocity at the take-off. At these moment the subject follow the sequence pattern of the lower limb rotation result in increase in amount of external force.
which applied to overcome the inertia and accelerate the body vertically. As the ground force increases the jump height increases\(^{[13]}\). In previous study they found out that sprint training produces similar or greater training effects than does plyometric training\(^{[2]}\).

Several studies showed that muscle power and overall muscle strength correlate negatively with the obesity in children and positively in adults with functional status and bone health. Even they suggest that there are many factor which can contribute to the decrease in the performance in relation to the horizontal jump like changes in the body and lifestyle which can be improved. Further studies should include the invasive technique that dependent on the individual’s subjectivity and cultural factors for proper use\(^{[9]}\).

In present study we find out that the maximum age group is around 21-25 years where they have achieved the maximum level of maturity. Even there is negative correlation of the age with both result of vertical and horizontal jump height. In previous study they stated that during and following the puberty there has been seen marked difference and unable to explain the absence of significant improvement \(^{[5]}\).

The result of the present study showed that there is significant effect of the training in recreational athletes indulging in jumping activities which increases both vertical and horizontal jump height of the players \((p=0.0001)\). Loturco et al. observe a meaningful increase in vertical and horizontal performances of elite U-20 players who executed short-term program for 3-week composed of vertical or horizontal. The reason behind this is the player’s both horizontal and vertical jump height being fully related to the body’s vertical as well as horizontal acceleration; and the acceleration is equal to force divided by mass. Sprint training increased the body’s vertical as well as horizontal acceleration thus increasing the capacity to accelerate the player’s own body weight. \(^{[13]}\)

Even in this study we found out the positive result of the athletes years of playing in relation with both vertical jump height week 1 \((p=0.86)\) and week 6 \((p=0.43)\) and horizontal jump height week 1 \((p=0.23)\) and week 6 \((p=0.05)\) which shows that have increased their performances for competitive level. This study has some limitation but majorly due to small sample size. Further studies can be done on larger sample size and also on the horizontal jump height as there are very rare studies done.

**Result**

There is significant comparison between the pre and post treatment \((p=0.0001)\) of horizontal and vertical jump height. Even there is positive correlation of years of playing in week 1 \((p=0.31)\) and week 6 \((p=0.58)\) for horizontal jump height and in week 1 \((p=0.86)\) and week 6 \((p=0.43)\) for vertical jump height but we found out that there is negative correlation of age in week 1 \((p=0.326)\) and week 6 \((p=0.002)\) of horizontal jump height and week 1 \((p=0.64)\) and week 6 \((p=0.31)\) of vertical jump height.

**Conculsion**

This study concluded that the effect of plyometric training was seen in recreational atheletes indulging in jumping activities.

**Acknowledgement:** We acknowledge the guidance and support from faculty of physiotherapy.

**Conflicts of Interest:** There is no conflict of interests in this study.

**Ethical Clerance:** This study has undergone ethical clearance through the university level ethical committee of Krishna institute of medical science, deemed to be university, Karad.

**Funding:** This study was self-funded.

**References**


Risk factors in Implant Placement: A Retrospective Analysis

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Abstract

Purpose: The aim of this retrospective study was to determine the prevalence of implant failure and its associated risk factors using a single implant system in our clinical setting.

Method: Patients who received implant treatment with a single implant system (MIS, Confident India) at Manipal College of Dental Sciences, Mangalore from 2010 to 2016 were enrolled. The following data were collected for analysis: patient details, field of expertise to which the surgeon belonged, diameter and length of the implant, The outcomes assessed were early or delayed failure on the basis of operator, implant, anatomy and patient related factors.

Results: This study analyzed 363 implants in 327 patients, who comprised 136 females and 191 males and were followed up until failure was reported after implant placement. 22 implants failed prior to final prosthesis delivery (early implant failure), and 5 implants were lost after prosthesis delivery. Out of 363 implants 206 (57%) implants were placed by the Department of Prosthodontics, 77 (21%) by the Department of Periodontics and 69 (19%) by the Department of Oral surgery and remaining were placed by a single Endodontist (3%) specialized in Implantology. (9/206=4%) failures were reported from Prosthodontics and 18 (18/69=26%) failures from Oral Surgery No failures were reported from Periodontics and Endodontics.

Conclusions: Prospective studies are warranted to further elucidate the factors contributing to implant failure. In the meantime, surgeons should receive appropriate training and carefully select the bone bed in order to minimize the risk of implant failure.

Key words: Early implant failure, overload, risk factors

Introduction

Dental implants have been considered to be a highly predictable treatment modality for replacing lost teeth in both partially and completely edentulous patients. Buser et al. retrospectively evaluated 511 implants with a sandblasted, large-grit, and acid-etched (SLA) surface, and reported a high 10-year success rate of 97%. However, not all implants are expected to show successful results, and adverse results that lead to implant removal are inevitable in routine practice due to the potential presence of many contributing factors.

Implant failure has been categorized as early and late in retrospective studies according to the time of prosthesis connection and time of loading. Recent studies have found that the prevalence of implant failure is higher in the early phase than in the late phase regardless of the loading time. This might be largely attributed to the vulnerability of the early phase, during which the primary stability decreases while the secondary stability gradually increases. Therefore, considerable interest has emerged in investigating the mechanism of early implant failure and the management interventions that are required to minimize the rate of implant failure.

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Early failure arises from a lack of osseointegration and that the clinically discernible mobility of an implant is a clear sign of early failure.5

Considering that dental implants are placed in a complex biological environment, multiple factors could contribute to implant failure. Numerous retrospective studies have shown that several patient-related, surgeon-related, and biomaterial-related factors can contribute to implant failure.6 However, many of these studies used diverse implant systems with different designs, which could have greatly affected the results.

Therefore, the aim of this retrospective study was to identify the factors influencing early implant failure at a single center using a single implant system.

Materials and Method

Inclusion of participants

This study recruited patients who received an implant-supported prosthesis using a single bone-level implant system (MIS, Confident India) with internal hex design and an SLA surface from 2010 to 2016 at this Institution. Patients with systemic disease were included if their condition was not a contraindication for implant surgery. Patients who were lost to follow-up and contact after implant placement were excluded from the study.

Clinical procedures

All implant placement procedures were mainly carried out by 18 residents supervised by 4 faculty members except those performed by the Endodontist. Sinus-floor elevation (SFE) and/or guided bone regeneration (GBR) was performed in patients with insufficient vertical bone height and/or width of the ridge crest either prior to or combined with the implant placement procedure. Either a 1-stage or 2-stage protocol of implant placement was selected according to the primary stability of the implant or the necessity for a bone augmentation procedure. In the 2-stage protocol, the second-stage surgery for implant exposure was performed after 3–4 months of healing.

All surgical procedures were performed under local anesthesia induced by 2% lignocaine hydrochloride with 1:200,000 epinephrine (Lox, Neon Laboratories, Mumbai, India). Sutures were generally removed at 7 days after surgery. The patients were recalled after 4–6 months, and the stability of implants checked clinically and then referred to the Department of Prosthodontics.

This study defined early implant failure as an implant showing clinical mobility before placement of a final prosthesis and late failure as clinical mobility after placement of final prosthesis with loading. Failed implants were removed after receiving consent from the patient, and the sites were restored using new implants or another prosthesis according to the modified treatment plan. Implants were categorized according to their size, the jaw in which they were placed (maxilla or mandible) and their position in the dental arch (anterior or posterior). The surgeons were dichotomized into faculty members and residents.

Results

Table 1: Number of Implants placed by different specialities from 2010-2016.

<table>
<thead>
<tr>
<th>Year</th>
<th>Periodontics</th>
<th>Prosthodontics</th>
<th>Oral Surgery</th>
<th>Conservative Dentistry</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>10</td>
<td>8</td>
<td>3</td>
<td>1</td>
<td>22</td>
</tr>
<tr>
<td>2011</td>
<td>4</td>
<td>20</td>
<td>6</td>
<td>1</td>
<td>31</td>
</tr>
<tr>
<td>2012</td>
<td>4</td>
<td>6</td>
<td>7</td>
<td></td>
<td>17</td>
</tr>
<tr>
<td>2013</td>
<td>14</td>
<td>25</td>
<td>22</td>
<td>1</td>
<td>62</td>
</tr>
<tr>
<td>2014</td>
<td>18</td>
<td>53</td>
<td>21</td>
<td>2</td>
<td>94</td>
</tr>
<tr>
<td>2015</td>
<td>20</td>
<td>34</td>
<td>7</td>
<td>5</td>
<td>66</td>
</tr>
<tr>
<td>2016</td>
<td>7</td>
<td>60</td>
<td>3</td>
<td>1</td>
<td>71</td>
</tr>
<tr>
<td>Total</td>
<td>77</td>
<td>206</td>
<td>69</td>
<td>11</td>
<td>363</td>
</tr>
</tbody>
</table>
Table 2: Number of failures recorded from 2010-2016

<table>
<thead>
<tr>
<th>No of failures</th>
<th>Periodontics</th>
<th>Prosthodontics</th>
<th>Oral Surgery</th>
<th>Conservative Dentistry</th>
</tr>
</thead>
<tbody>
<tr>
<td>27/363 (7.4%)</td>
<td>--</td>
<td>9/206 (4.3%)</td>
<td>18/69 (26%)</td>
<td>--</td>
</tr>
</tbody>
</table>

Table 3 Number and Type of Failures

<table>
<thead>
<tr>
<th>Early Failures</th>
<th>Late Failures</th>
</tr>
</thead>
<tbody>
<tr>
<td>22 (6%)</td>
<td>5 (1%)</td>
</tr>
</tbody>
</table>

Table 4: Association between size of implant . position in the the jaw

<table>
<thead>
<tr>
<th>No of failures</th>
<th>Anterior maxilla D2/D3 Bone</th>
<th>Posterior maxilla D3/D4</th>
<th>Posterior mandible D2 Bone</th>
</tr>
</thead>
<tbody>
<tr>
<td>27/363</td>
<td>13 (48%)</td>
<td>7 (25%)</td>
<td>7(25%)</td>
</tr>
</tbody>
</table>

Discussion

This retrospective assessment of the factors contributing to early implant failure found that the early failure rate of a single implant system was 6% and late failure rate was1% with the total implant failure rate being 7.4%.with significant number of failures associated with residents of Oral Sirgery.

Highly predictable long-term results of oral rehabilitation using dental implants have been documented in efficacy studies performed over the past 50 years. Literature has assessed the effectiveness of implant therapy and analyzed risk factors for implant failure with the aim of further improving treatment outcomes. The current study also focused on the latter aspects, but only included patients who received implant treatment using a single implant system at a single center in order to minimize the possible confounding effects of the surface or system of the implant and other differences between implant brands. In addition, the present study focused on implant failure occurring between different specialties of dentistry and the incidence of early failure.

The prevalence of early failure in the present study was within the previously reported range at the implant level (1.3%–6.36%). Rates can vary with the cutoff time point used to divide the early and late phases. Setting the cutoff time as the time of abutment connection has the advantage of minimizing heterogeneity resulting from differences in the timing of loading and the loading methods. Meanwhile, setting the cutoff time as 1-year after final prosthesis delivery can reflect delayed failures suspected to have been initiated during implant surgery and the tendency for radiographic evaluations to be performed for evaluating marginal bone loss.

Implants placed in the anterior maxilla were found to have a higher risk of failure in this study 4 of which were on account of road traffic accidents and defeciency in premaxillary region requiring bone grafting and one was on account of immediate implant placement in an infected socket. Therefore, the higher risk of implant failure in the anterior maxilla should be interpreted in the context of the characteristics of patients as demonstrated in the present study.

This study found that the implant failure was higher for shorter 8-mm implants with most of these implants failing in the posterior maxilla( 75%). It is well known that the length of an implant has less effect than its
diameter on dispersing occlusal loads. In addition, it has been reported that the outcomes for short implants (less than 8 mm) appear to be as predictable as those for standard-length implants placed in augmented bone. Placing a short implant will reduce the degree of invasion into surrounding anatomical structures and the need for regenerative procedures. Most of these short implants were placed by residents in order to avoid invasive procedures of Direct as well as in some instances indirect sinus lift. A retrospective study found that the survival rate of implants did not appear to be associated with the experience of the 80 included surgeons. Meanwhile, another study found that a learning curve was present for implant placement, in that the early failure rate was 2-fold higher for surgeons who had previously placed fewer than 50 implants than for surgeons who had placed more than 50 implants. Experience or competence of the surgeon influences the failure rate of dental implants. Therefore, the results should be interpreted in context of the characteristics of competency-based surgical skill of residents and experience necessary for successful implant placement.

Jemt et al., who suggested that the individual personality or attitude of the surgeon can affect the implant outcome, based on the rate of implant failure being significantly lower for 1 surgeon who preferred a 2-stage protocol and delayed implantation after tooth extraction. This could be a possible explanation as to why most implants failed when speciality of Oral Surgery and Prosthodontics placed implants.

The main limitation of this study is that the medical histories of patients were not fully scrutinized. However, recent studies did not identify any medical conditions that significantly increased the risk of implant failure. Furthermore, eliciting and recording the medical history only based on the verbal statements of patients might provide incomplete or inaccurate information. Another limitation is that parameters regarding bone quality and quantity were not analyzed objectively. The effect of alveolar ridge atrophy on early implant failure can vary considerably according to the implant position and dimension. Therefore, it might be helpful for avoiding bias to use postoperative parameters such as peri-implant bone wall thickness, dehiscence, or fenestration size when analyzing bone quantity.

Recently developed implants have macro-level designs and surfaces that improve the primary stability and facilitate osseointegration. Nevertheless, implant failure is still observed in routine practice.

**Conclusion**

The present study found that speciality and experience of the surgeon, position of the bone bed of patients and height of the implant affected the likelihood of implant failure using a single-implant system. Considering that the characteristics of the surgeon strongly influenced early implant failure, educational programs should be developed for increasing surgical competency and performance, and further prospective studies are warranted.

**Conflict of Interest**: Nil

**Source of Funding**: Self

**References**

7. Buser D, Sennerby L, De Bruyn H. Modern implant


Legends
Effect of Upper Body Strength Training in Spatiotemporal Parameters of Gait In Individual with Thoracic Kyphosis

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Abstract

Background: The human spine is an integral part of the human body. Its function includes mobilizing the torso, controlling postural stability, transferring, loads from upper body to lower body, all of which is essential for the activity of daily living. Spine deformity affects spinal mobility and trunk balance, thus altering locomotion pattern during each step. Spinal deformity also alters the center of body mass (com) during gait which leads to gait abnormality. Exaggerated thoracic kyphosis is common in the elderly as there is age related increase in thoracic spine curvature. Generally, patients with thoracic kyphosis mostly have gait impairment with reduced movement in upper limb strength and muscle mass gets altered in upper limb.

Objective: The objective of the study is to study the effect of upper body strength training on spatiotemporal parameters of gait in individuals with thoracic kyphosis and to find out the effect of upper body strength program on gait parameters after thoracic kyphosis.

Material and Method: In this interventional study a total of 25 individuals with in the age group of 20 to 50 years, were selected. Pre-test and post-test assessment was done using the outcome measures of flexi curve ruler to assess kyphosis index and spatiotemporal measurement. Exercises were administered for 6 weeks. Later, post-test assessment and statistical analysis was done to determine its significance.

Results: According to the result, there was a considerable change in the stride length(p-value<0.0001) step length(p-value<0.0001) and cadence (p-value<0.0001). There was also a marked change in the kyphosis index. (p-value<0.0001).

Conclusion: As per the result of this study, we conclude that the effect of upper body strength training on spatiotemporal parameters of gait in individuals with thoracic kyphosis was extremely significant.

Keywords: Kyphosis, strength, thoracic, spatiotemporal, gait, parameters, upper body, stride length, step length, cadence.

Introduction

Thoracic kyphosis refers to forward curvature of the thoracic spine. Exaggerated thoracic kyphosis is common in the elderly as there is age related increase in the curvature of the thoracic spine¹.

There are three main types of abnormal kyphosis. Postural kyphosis is more common in girls than in boys and typically appears during adolescence. Poor posture and weakening of the muscles and ligaments in the back causes postural kyphosis². This patient can have symptoms of pain and muscle fatigue. Scheuermann’s kyphosis is the result of structural deformity of the vertebrae. It is more common to develop kyphoscoliosis with Scheuermann’s kyphosis than with any other type of kyphosis. Congenital kyphosis is the least common

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type of abnormal kyphosis and occurs due to abnormal development of vertebrae.

The most common symptom for patients with abnormal kyphosis are appearance of poor posture with a hump at the back or round back. Symptoms may also include back pain, muscle weakness and stiffness in the back. Physical and social outcomes of spinal kyphosis include in high, protuberant abdomen, downward gaze, digestive problems, impairments of respiratory function and decreased quality of life. Therefore, basic activates of daily living, leisure, and emotional are negatively affected by spinal deformity. The functional consequence of this postural deformity and the overall decline in quality of life often overlook. Generally, patients with thoracic kyphosis have gait impairment with reduced movement in upper limb. Reduction in strength and muscle mass in upper limb also occurs.

Gait is the most common human movement involved in daily activity. Gait is the series of alternating movement of the lower extremities in a rhythmic motion that results in forward propagation of body. Gait parameters like cadence, step length and stride length generally tend to be reduced due to thoracic kyphosis. Basically cadence is the number of steps taken per unit time. Step length is the distance between corresponding successive points of heal contact of opposite feet. Stride length is the distance between successive points of heal contact of the same foot.

Normally in patients with altered gait, free movement of upper limb is necessary to improve gait and its parameters. Upper limb strengthening also helps in free motion of upper limb.

The human spine is the integral part of the human body. Its function includes mobilizing the torso, controlling postural stability, transferring, loads from upper body to lower body, all of which is essential for the activity of daily living. Spine deformity affects spinal mobility and trunk balance, thus altering locomotion pattern during each step. Spinal deformity changes the center of body mass (COM) during gait which leads to gait abnormality.

Measurement should be taken on the same population for which the measure is intended. For assessment of severity of kyphosis, normally radiographic method is used. However, it is a relatively expensive method as well as exposes the individual to radiation. Hence, flexi curve ruler is used here. It is a flexible ruler that molds according to the curvature of the spine and thus the angle can be measured. It reliability had a intra class correlation coefficient of 0.906 which is highly reliable.

Therefore, the purpose of this study was to assess the pre-test and post-test reliability of the measurement of thoracic kyphosis using the flexi curve ruler with spatiotemporal parameters measurement.

Methodology

The ethical clearance was taken from ethical committee of Krishna institute of medical sciences, Karad. There were 25 participants in the study. The study took place in the Physiotherapy department, Krishna institute of medical sciences. The study was effect of upper body strength training in spatiotemporal parameters of gait in individual with thoracic kyphosis. The treatment protocol was of 8 weeks. The subjects were assessed for severity of kyphosis and spatiotemporal parameters as well. The type of study was experimental study. The study design was pre and post. The samples were chosen using the simple random sampling method. Participants were included as per inclusion criteria, i.e. age group of 20-50 years, individuals with acquired thoracic kyphosis and with Secondary kyphosis.

Procedure

All the subjects were selected for the study according to the selection criteria. Demographic data and consent form was provided. Pre and post assessment were taken before the treatment began and after completion of treatment i.e. at 6 weeks respectively with the help of outcome measures.

In 1st and 2nd week, mirror image thoracic extension exercises for a hold time of 30sec and for a total of 5 repetitions was given. Along with this, head retraction for a hold time of 15sec and for a total of 5 repetitions was given as well as superman exercise for a hold time of 3sec and for a total of 10 repetitions was given.

In 3rd and 4th week, mirror image thoracic extension exercises for a hold time of 30sec and for a total of 7 repetitions was given. Along with this, head retraction for a hold time of 15sec and for a total of 5 repetitions was given as well as superman exercise for a hold time of 3sec and for a total of 10 repetitions was given.

In 3rd and 4th week, mirror image thoracic extension exercises for a hold time of 30sec and for a total of 7 repetitions was given. Along with this, head retraction for a hold time of 15sec and for a total of 7 repetitions was given as well as superman exercise for a hold time of 3sec and for a total of 15 repetitions was given. Raise up thoracic extension for a hold time of 15sec and for a total of 10 repetitions was given.
In 5th and 6th week, mirror image thoracic extension exercises for a hold time of 30sec and for a total of 10 repetitions was given. Along with this, head retraction for a hold time of 15sec and for a total of 10 repetitions was given as well as superman exercise for a hold time of 3sec and for a total of 15 repetitions was given. Raise up thoracic extension for a hold time of 15sec and for a total of 15 repetitions was given along with thoracic spine foam rolling for a hold time of 30sec and for a total of 10 repetitions was given.

Results and Discussion

1) Age wise distribution

Table 1: Distribution of kyphotic individuals according to age

<table>
<thead>
<tr>
<th>Age Group</th>
<th>No. of Individuals</th>
<th>Percentage</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-30</td>
<td>13</td>
<td>52.0%</td>
<td>23.93±2.72</td>
</tr>
<tr>
<td>31-40</td>
<td>8</td>
<td>32.0%</td>
<td>36.37±3.34</td>
</tr>
<tr>
<td>41-50</td>
<td>4</td>
<td>16.0%</td>
<td>45.5±2.65</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>100.0%</td>
<td>31.36±8.92</td>
</tr>
</tbody>
</table>

**Interpretation:** According to table no. 1, 13 (52%) individuals belong to the age group (21-30) years with a mean age of 23.93 years, 8 (32%) individuals belong to the age group (31-40) years with a mean age of 36.37 years, 4 (16%) individuals belong to the age group (41-50) years with a mean age of 31.36 years.

2) Distribution according to gender

Table 2: Distribution according to gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>No. of Individuals</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>16</td>
<td>64.0%</td>
</tr>
<tr>
<td>Female</td>
<td>9</td>
<td>36.0%</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

**Interpretation:** According to table no.2, 16 (64%) individuals were male and 9 (36%) individuals were female.

3) Distribution of kyphosis index scores

Table 3: Distribution of kyphosis index scores pre and post intervention respectively.

<table>
<thead>
<tr>
<th>Kyphosis index</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>35.92±4.99</td>
</tr>
<tr>
<td>Post</td>
<td>33.44±5.51</td>
</tr>
</tbody>
</table>

**Interpretation:** According to table no.3, the mean score for kyphosis index prior to intervention was 35.92 and post intervention was 33.44.
4) Association between kyphosis index pre and post intervention

**Table 4: Association between kyphosis index scores pre and post intervention respectively.**

<table>
<thead>
<tr>
<th>Kyphosis index</th>
<th>Paired t-test</th>
<th>t- value</th>
<th>p- value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t- value</td>
<td></td>
<td>p- value</td>
</tr>
<tr>
<td></td>
<td>8.667</td>
<td></td>
<td>&lt;0.0001(ES)</td>
</tr>
</tbody>
</table>

**Interpretation:** According to table no. 4, the association between pre and post intervention scores of kyphosis index was compared using paired t-test with a p-value of <0.0001(ES).

5) Distribution of spatiotemporal parameters pre and post intervention

**Table 5: Distribution of spatiotemporal parameters pre and post intervention**

<table>
<thead>
<tr>
<th>Spatiotemporal Parameters</th>
<th>Mean±SD</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stride Length</td>
<td>Step Length</td>
<td>Cadence</td>
</tr>
<tr>
<td>Pre</td>
<td>68.48±6.96</td>
<td>34.32±3.36</td>
<td>76.32±9.53</td>
</tr>
<tr>
<td>Post</td>
<td>75.56±9.48</td>
<td>37.76±4.74</td>
<td>80.88±10.91</td>
</tr>
</tbody>
</table>

**Interpretation:** According to table no. 5, the mean score for stride length pre and post intervention was 68.48 and 75.56 respectively, for step length pre and post intervention was 34.32 and 37.36 respectively, and for cadence pre and post intervention was 76.32 and 80.88 respectively.

6) Association between spatiotemporal parameters pre and post intervention

**Table 6: Association between spatiotemporal parameters pre and post intervention**

<table>
<thead>
<tr>
<th>Spatiotemporal Parameters</th>
<th>Paired t-test</th>
<th>t- value</th>
<th>p- value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t- value</td>
<td></td>
<td>p- value</td>
</tr>
<tr>
<td></td>
<td>8.752</td>
<td></td>
<td>&lt;0.0001(ES)</td>
</tr>
<tr>
<td></td>
<td>7.502</td>
<td></td>
<td>&lt;0.0001(ES)</td>
</tr>
<tr>
<td></td>
<td>6.979</td>
<td></td>
<td>&lt;0.0001(ES)</td>
</tr>
</tbody>
</table>

**Interpretation:** According to table no.6, association between spatiotemporal parameters was done using paired t-test. Stride length had a p-value of <0.0001(ES), step length had a p-value of <0.0001(ES) and cadence had a p-value of <0.0001(ES).

**Discussion**

The human spine is the integral part of the human body. Its function includes mobilizing the torso, controlling postural stability, transferring loads from upper body to lower body, all of which is essential for the activity of daily living. Spine deformity affects spinal mobility and trunk balance, thus altering locomotion pattern during each step. Spinal deformity changes the center of body mass (com) during gait which leads to gait abnormality. Exaggerated thoracic kyphosis is common in the elderly this age related increase in thoracic spine. Generally patient with thoracic kyphosis mostly have gait impairment with reduced movement in upper limb strength and muscle mass gets altered in upper limb. Thus kyphotic individuals need appropriate treatment and awareness of such therapeutic techniques
like upper body strengthening necessary for preventing further complications as well as improving the quality of life of the individual.

In this study, 25 individuals who had kyphosis were taken. Individuals in the age group of 21-50 years were included in this study, out of which 13 (52.0%) individuals were in the (21-30) age group, 8 (32.0%) individuals were in the (31-40) age group, 4 (16.0%) individuals were in the (41-50) age group.

Also, out of the total 25 individuals participating in this study, 16 (64%) were male and 9 were female (36%).

Score according to Kyphosis index (KI) and spatiotemporal parameters of subjects were taken before the treatment/intervention was given. The mean Kyphosis Index score prior to the intervention was 35.92 and post intervention was 33.44.

Statistical analysis of Kyphosis index score of pre and post intervention was done to confirm that the difference between the pre and post measurements is significant. The comparison for pre and post Kyphosis index scores was found to have a p-value of (<0.0001) which was extremely significant.

To analyze the impact of kyphosis on gait in terms of spatiotemporal parameters like stride length, step length and cadence was done. Same as Kyphosis index score, measures of stride length, step length and cadence were taken before and after the treatment. The mean scores of stride length pre and post intervention was 68.48 and 75.56 respectively, for step length the pre and post intervention score was 34.32 and 37.76 respectively and for cadence the pre and post intervention score was 76.32 and 80.88 respectively. Here, stride length was altered the most among all the parameters.

Statistical analysis was done to compare the mean pre and post intervention scores of spatiotemporal parameters to confirm whether they were significant or not and it was found that stride length had a p-value of (<0.0001) which was extremely significant, step length had a p-value of (<0.0001) which was extremely significant and cadence also had a p-value of (<0.0001) which was extremely significant. Thus there was a significant improvement in the spatiotemporal parameters post intervention.

The reason behind this may be due to the fact that the exercises administered by us also utilized the lower limb muscles in some instances. Moreover, these exercises are known to strengthen the scapular muscles as well as the back muscles that link the trunk to the lower extremities. The trunk is the central key point of the body, and the control of movement proceeds from proximal to distal body regions. Motor control literature also suggests that an improvement in the levels of proximal trunk control might lead to a better distal limb control. Hence, strengthening the trunk plays a significant role in improving gait.

Thus the results obtained by studying both the pre and post intervention scores in this study suggest that in treatment of kyphosis, upper body strengthening is extremely effective.

Acknowledgement: I sincerely thank the management of KIMSDU for allowing me to conduct this study by providing me the necessary requirements. I thank my Dean Dr. G. Varadharajulu sir for his support and guidance. I convey sincere thanks to my guides Dr. Poonam Patil for helping me in my research. I take this opportunity to thank all those who have been directly or indirectly involved for smooth conduction of my study.

Conclusion

On the basis of the result of the study it is concluded that in treatment of kyphosis, upper body strengthening is extremely effective.

Source of Funding: Source of Funding: Krishna Institute Of Medical Sciences Deemed to be University, Karad.

Conflict of Interest: There were no conflicts of interest in my study.

Ethical Clearance: The Institutional Ethical Committee has hereby given permission to initiate the research project titled, “Effect of upper body strength training in spatiotemporal parameters of gait in individual with thoracic kyphosis.”

References


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Factors Related to Alzheimer’s Disease, Tau Pathology in Alzheimer’s Disease: Possible Treatments for Tau Pathology

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Abstract

The Tau protein is a microtubule-associated protein that acts as a three-dimensional “railroad tie” for the microtubule. Accumulation and Aggregation of the Tau is the pathogenesis of Alzheimer’s disease. Risk factors like ApoE risk alleles, changes in the endoplasmic reticulum, and Kinases and phosphatases dysregulation have identified as the most critical factors. In Tau pathology, the abnormal hyperphosphorylation of tau appears as its accumulation in the affected neurons in Alzheimer’s disease.

Neurofibrillary Tangles has shown truncated tau in both Glu-391 and Asp-421. Truncated tau associated with apoptosis in cultured cells. All six molecules of tau are the hyperphosphorylated state in PHF. In AD, hyperphosphorylated tau is present as a cytosolic protein and PHF. Treatments related to tau pathology are under research. Tau phosphorylation inhibitors and Tau aggregation inhibitors tested in people with AD. In tau phosphorylation inhibitors, Lithium has multiple targets and inhibits GSK-3b, and in tau aggregation inhibitors, many drugs block aggregation of tau in cell-free conditions. Methylene blue has multiple targets; it slows disease progression. Tau pathology appears to be a primary cause of neurodegeneration in AD. Risk factors showed a relation between AD and Tau pathology clearly. Abnormal hyperphosphorylation of tau leads to AD, and truncated tau is the main finding in tau pathology. Tau phosphorylation inhibitors and Tau aggregation inhibitors are emerging treatments.

Keywords: Alzheimer’s disease, Paired Helical Filaments, Neurofibrillary tangles, Tau pathology, Abnormal Hyperphosphorylation, Truncation, Conformation and Isoforms, Toxicity, Tau Phosphorylation Inhibitors and Tau Aggregation Inhibitors.

Introduction

Tau is the essential Microtubule-Associated proteins (MAP) in the neurons and acts as a three-dimensional “railroad tie” for the microtubule. Accumulation and Aggregation of the Tau is the pathogenesis of Alzheimer’s disease. Phosphorylation of tau binds it to the microtubules and helps in maintaining the structure, stability of neurons¹. Accumulation of phosphate (Hyperphosphorylation) on the tau proteins cause “paired helical filaments” (PHFs) that accumulate and lead to the neurofibrillary tangles (NFTs)². PHFs are the main component in NFTs. Abnormally hyperphosphorylated, insoluble, and filamentous tau was the main component of Neurofibrillary Tangles³⁴⁵. NFTs are neurological hallmark of AD⁶. It expressed in the central and peripheral nervous system and less amount observed in the kidney, lungs, and testis⁷. Abundantly seen in neuronal axons⁸. The human tau gene is located over 100 kb on the long arm of chromosome 17 at band position 17q21 and has 16 exons⁹. Tau divided into four regions: N-terminal Projection Region, a Proline-Rich Domain, a Microtubule-Binding Domain (MBD), a C-terminal region¹⁰. Tau can bind to outside and inside of microtubules by N- and C-terminal regions projecting outwards¹¹¹².
Factors related to Alzheimer’s disease

ApoE Risk alleles

ApoE is a risk factor related to AD in relation to tau pathology. ApoE, as a vital component of chylomicrons and IDLs involved in the catabolism of lipoproteins. It has a crucial role in the metabolism of fats in the body. ApoE is a cholesterol carrier in the brain. ApoE transfers lipoproteins, fat-soluble vitamins, cholesterol to the lymph vessels, and into the blood. ApoE is abundant in CNS but synthesized in the liver.

Accumulated cholesterol in the neurons converted into 24OHC (24S-hydroxycholesterol) in neurodegeneration. The CSF tau levels related to neurodegeneration in AD. The association between ApoE, 24S-hydroxycholesterol, and tau shows its direct involvement in the generation of NFTs.

Damage in Endoplasmic reticulum

Damage in Endoplasmic reticulum is a significant risk factor related to AD in relation to tau pathology. The Endoplasmic reticulum functions are energy production, folding, and trafficking of proteins and apoptosis. Role of ER in the development and progression of neurodegenerative diseases. Inositol-Requiring kinase 1 (IRE1) starts the ER stress pathway by triggering Apoptosis signal-regulating Kinase 1 (ASK 1), activates c-Jun N-terminal Kinase (JNK) signaling route. This cascade has the strength to trigger AD pathology through hyperphosphorylation of tau and aggregation of neurofibrillary tangles. ER stress causes deposition of misfolded like tau, and long-time existence of these toxic misfolded proteins triggers intrinsic apoptosis pathways. Overexpression increase the intracellular concentration of tau may inhibit the plus-end-directed transport of vesicles along microtubules by kinesin, and the minus-end-directed transport by dynein becomes dominant.

Tau degradation decreased by 20% in ER stress due to a decrease in the tau binding to CHIP (carboxyl-terminus of Hsc70-interacting protein), which delayed the tau degradation through the ubiquitin-proteasome pathway.

Kinases and phosphatases dysregulation

Dysregulation of kinases and phosphatases is a critical risk factor related to AD in relation to tau pathology. Several protein kinases like cyclic AMP-dependent Protein Kinase A (PKA), Glycogen Synthase Kinase-3 (GSK-3), cyclin-dependent protein kinase-5 (cdk5), Calcium/Calmodulin-dependent Protein Kinase-II (CaMKII), Mitogen-Activated Protein (MAP) kinase, Extracellular signal-regulated kinase (ERK 1/2), Stress-activated protein kinases are involved in abnormal hyperphosphorylation of tau. Some kinases like Protein Kinase A (PKA), Protein Phosphatases 2A (PP2A), Glycogen Synthase Kinase-3 (GSK-3), Calcium/Calmodulin-dependent Protein Kinase-II (CaMKII) plays a role in Tau phosphorylation and dephosphorylation. Abnormally hyperphosphorylated sites in tau are proline-directed, serine, and threonine, followed by proline are official sites of proline-directed protein kinases (PDPKs). GSK-3, cdk5, PDPKs, and ERK 1/2 are subjected to phosphorylate tau the sites in AD.

The preparation of tau by PKA or CAMKII is enough to initiate the abnormal hyperphosphorylation of tau. Dysregulation of Tau kinase CAMKII effects AD progression, and CAMKII inhibits tau-microtubule interaction by tau phosphorylation. When GSK-3 overexpressed and it results in hyperphosphorylation of tau. GSK-3 activation results in brain aging and AD initiates detrimental events like NFT formation and neuronal death pathways. PP-2A and PP-1 do 90% of serine/threonine protein phosphatase activity. Phosphorylation of ERK inhibits activity in response to neuronal stimuli.

Tau pathology

In AD, the regular role of tau impaired because this protein loses its capacity to bind to microtubules. Tau pathology starts from the entorhinal cortex and continues to the hippocampus, neocortex, frontal, temporal cortices, and all isocortex areas. Tau pathology observed in the form of abnormal Hyperphosphorylated protein. There is a very robust correlation between tau pathology and clinical measures of dementia. In AD, tau pathology also plays a significant role because this has the potential to trigger AD in humans.

Abnormal hyperphosphorylation of tau

The main characteristic feature of tau pathology is the abnormal phosphorylation of tau. The abnormal hyperphosphorylation of tau and significant protein subunit of PHF, which results in neurodegeneration by sequestration of MAPs, self-assembles bundles of
PHF and forms NFTs. In Tau pathology, accumulation of abnormal Hyperphosphorylated tau associated with neurofibrillary degeneration and dementia. The abnormal hyperphosphorylation of tau observed in both NFTs and the cytosol of AD brains. Mutations in the tau gene and their cosegregation linked to chromosome-17 (FTDP-17) have abnormalities in tau as a leading, and the first event leads to neurodegeneration and dementia. The study of mAb Tau1 revealed deposits of abnormally hyperphosphorylated tau in neurons without tangles (stage “0” tangles) in AD. In tau, Ghost tangles are ubiquitinated, and in abnormally hyperphosphorylated tau, cytosome has no ubiquitin reactivity. Abnormal hyperphosphorylation of tau might be due to a conformational change in tau, and tau conformationally altered in AD. On hyperphosphorylation, murine tau self assembles into tangles of filaments (PHF/ SF)\(^4\). Abnormal hyperphosphorylation of tau comes before its accumulation into NFT’s. In our research, tau hyperphosphorylation seen at many sites and even HMW-tau hyperphosphorylated at many locations. An essential finding of the study is overexpressing p25 initiates and promotes hyperphosphorylation. This abnormal hyperphosphorylation leads to filaments self-assembly.

**Truncated tau**

Presence of truncation connected to neurofibrillary pathology in the brain of AD patients. Neurofibrillary Tangles has shown truncated tau in both Glu-391 and Asp-421. Truncated Tau associated with apoptosis in cultured cells. There is no report on what percentage of tau truncated at affected sites in different stages of AD. Neurodegeneration in AD seen for a long time from months to years, and there is a fair chance to view truncation in tau. Truncation is seen in both affected neurons and ghost tangles (extracellular space) when NFTs exposed to hydrolases. Truncation of tau was observed by immunolabeling with the monoclonal antibody Tau-C3. Tau-C3 recognizes Asp-421. Asp-421 was associated with the neurofibrillary pathology in the AD brain. In vitro studies described that truncated tau at ASP-421 showed elevated rates of polymerization over full-length tau. Cells transfected with truncated tau protein, it has demonstrated that both soluble and insoluble forms can induce toxicity. In COS7 cells that Asp-421 is highly phosphorylated and abnormally redistributed. In HEK-293T cells that Glu-391 has a problem in binding with microtubule, and there is toxicity resulted in cell death. In the progression of the disease, Early event truncation of Asp-421 and preceded by truncation of C-terminal of Glu-391, further occurring from intermediate to advanced stages of NFTs evolution. In truncation, this research specially confined to truncated tau in Glu-391 and Asp-421. In our findings, COS7 cells highly phosphorylated in Asp-421 and HEK cells have a binding problem with microtubule.

**Isoforms of tau**

Six isoforms of tau differ by contents of three (3R) or four (4R) tubulin-binding domains of 31 or 32 amino acids in C-terminal and one (1R), two (2R) or inserts of 29 amino acids each in N-terminal of tau. All six molecules of tau are the hyperphosphorylated state in PHF 3,4,54. 441-residue tau is the longest tau isoform seen in the human CNS. In AD, hyperphosphorylated tau is present as a cytosolic protein and PHF 3,4,54. Cytosolic hyperphosphorylated tau (P tau) has 5-9 mol of phosphate per mole of the protein (contains 2-3 phosphate groups). Tau self-assembles by intermolecular hydrophobic interaction and microtubule-binding repeat R3 (3R taus), and R2 and R3 (4R taus), when the rest of the molecule neutralized. Inhibitory amino-terminal and carboxyl-terminal regions neutralized by abnormal hyperphosphorylation in AD. Cytosolic and PHF are abnormally hyperphosphorylated taus are readily dephosphorylated by phosphatases in vitro. Tau molecule changing its conformation to form a paperclip-like conformation by folding the N- and C-terminal portions back on the microtubule-binding repeats. FTDP-17 mutations alter the conformation of the protein, and it becomes a more favorable substrate to brain protein kinases. Mutated taus are soon hyperphosphorylated and self-assemble at a lower level of hyperphosphorylation. In tau, Dephosphorylation inhibits self-assembly, and hyperphosphorylation promotes self-assembly, but Deglycosylation of AD promotes self-assembly. This study found that all six tau
isoforms self-assembled into PHF/SF. This result clearly explains that hyperphosphorylation is enough to initiate self-assembly of tau into filaments. The 3R isoforms are more toxic than 4R.

**Possible treatments for tau pathology**

Treatments targeting different aspects of tau pathology are under research. Tau phosphorylation Inhibitors and Tau Aggregation Inhibitors are in clinical trials for people with AD, while tau reduction strategies are still in preclinical trials.

**Tau Phosphorylation Inhibitors**

In tau phosphorylation inhibitors, Lithium has multiple targets and inhibits GSK-3β69. Lithium has a narrow safety margin60. Studies in geriatric patients taking chronic Lithium for BPAD showed a reduced risk of developing AD. Lithium inhibits chemical changes in tau that lead to the formation of NFT’s. Studies on Lithium shown benefit with low doses in mild cognitive impairment, Reduction of GSK-3β impairs NMDAR-mediated long-term depression61 and memory consolidation62, raising concerns about potential side effects of GSK-3β inhibitors. CDK5 is essential for multiple cell signaling pathways and adult neurogenesis, limiting its appeal as a tau-targeting approach in AD. However, CDK5 and p25, a truncated form of the CDK5 subunit p35, promote neurodegeneration through mechanisms that are independent of tau phosphorylation63.

**Tau Aggregation Inhibitors**

Filamentous tau aggregates are damaging forms of tau and Filamentous tau is toxic. Tau enters dendritic spines, and adverse effects of tau aggregates are seen only in intracellular compartments64. Many of the drugs that block the aggregation of tau also prevent the pathological aggregation of other proteins under cell-free conditions265. Some tau aggregation inhibitors are effective in Neuro2A cell lines overexpressing a 4R tau microtubule repeat domain fragment with a K280 deletion and promotes its aggregation66. In AD patients, the Methylthioninium Chloride (methylene blue) showed slowing disease progression67. Methylene blue is the first drug targeting tau. The drug derived from the dye used to stain NFT’s in neuropathological studies. Primarily inhibits tau aggregation and showed cognitive benefits. Methylene blue inhibits tau-tau interactions68 and reduces soluble tau by mechanisms69. Inhibition of tau aggregation mediated by direct binding of tau to the FK506 binding protein 5270. It is unknown that tau assembly is responsible for tau-dependent neuronal dysfunction and degeneration71.

**Other treatments**

Hyperacetylation increases half-life72 and due to this microtubule-binding impairs and initiates aggregation73. Lysosomal pathway degrades proteasome, removes aggregated tau74, and inhibition of this pathway produces NFT-like tau deposition75. Lysosomal pathway of tau degradation involved in Niemann-Pick type C disease (NPC), seen with neurological symptoms and NFT formation76. NPC caused by loss of function of NPC1 (Lysosomal trafficking protein)77. We found that that degradation of tau occurs in Lysosomal and ubiquitin-proteasome pathways. Destabilization of microtubules and problem binding to microtubules are two main problems related to overexpression of tau. Microtubule stabilizers have shown excellent results in preclinical and clinical trials of AD. Epothilone D has better BBB permeability, improved microtubule density, and cognition78. Peptide NAP stabilizes microtubules79 and reduces tau phosphorylation80. In our study, Microtubule stabilizers have shown more than one mechanism of action.

**Conclusion**

Tau pathology is the leading and primary cause of neurodegeneration in AD. In the brain of AD, the level tau expression has no change concerning healthy aging, and toxicity depends on the contribution of many factors, like loss of normal function, the impermanent aggregation, and state of tau processing. Abnormal posttranslational modifications, like hyperphosphorylation and truncation, are responsible for altered tau structure in AD. Abnormal hyperphosphorylation and truncation are supported by *in vitro* experiments explain modifications elevate fibrillization of tau and induce cell toxicity81. Conformational changes of tau promoted by posttranslational modifications and the role of fibrillization important for checking the potential of tau-directed therapies81. Inhibition of the abnormal Hyperphosphorylation of tau and sequestration of MAPs by the hyperphosphorylated tau41 is the most critical therapeutic targets for AD. The pathogenic tau slowed or prevented by treatments that reduce levels of extracellular tau, so neurons cannot internalize it82. Identification and characterization of mechanisms of tau release and uptake
are essential for therapeutic interventions that may slow or prevent neurodegeneration in AD. Drugs are needed to develop more effectively to slow or prevent tau pathology. Methods to be prepared to restrict or inhibit the hyperphosphorylation of tau because inhibiting abnormal hyperphosphorylation does not self-assemble tau, and there will be no formation of PHF/SF.

**Abbreviations**


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**Ethical Considerations:** Compliance with ethical guidelines

There is no ethical principle to be considered during this research.

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Association between Running Activity and Pronated Foot Posture in East Java Puslatda Athletes

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Abstract

Background: Athlete begins intense exercise at a young age when the musculoskeletal system is still immature, thus it has the potential to cause specific changes in body. A more pronated foot have a greater mobility in both lateral medial plane and vertical plane. Hyperpronated foot allegedly can increase injury risk because it will cause a great moment of force and instability. The research aimed to determine correlation between running activity and pronated foot posture, and to obtain foot posture description of East Java puslatda athletes during running activity in their exercise.

Methods: The research employed cross sectional design using 75 athletes, consisting of 43 athletes who run more than 20 km per week and 32 athletes who run less than 20 km per week. The research variables were running activity obtained from questionnaire and foot posture obtained from the Foot Posture Index examination. The study protocol was approved by the ethics committees of Dr. Soetomo Teaching Hospital (Surabaya, Indonesia).

Results: The subjects’ demographic data characteristics based on age and exercise duration showed no difference between the two sample groups. On the other hand, the research found a significant difference between age (p = 0.001) and body mass index (p = 0.049). There was no significant correlation between running activity and pronated foot posture, with p = 0.293.

Conclusion: The study found that running activity more than 20 km per week had no correlation with pronated foot percentage. Pronated foot posture was commonly found in the sample group with running activity more than 20 km per week, but it was not statistically significant.

Keywords: athlete, foot postur index, pronated foot posture, running.

Introduction

Athlete begins intense exercise at a young age when the musculoskeletal system is still immature, thus it has the potential to cause specific changes in body. Athlete’s foot morphology may be associated with a variety of sports. Some data suggest that specific exercise and repetitive movements in certain sports can affect foot arch index ¹. In his study in 1323 professional athletes, found an increased risk of flat foot and splay foot occurrences in the endurance runner group ².

A research conducted in General Practice Department of Groningen University and Rehabilitation Department of Utrechts University, Netherland, from September 2007-April 2009 found 23.7% of sport injury incidence per 1000 patients, with 27.8 prevalence per 1000 patients ³. Running-related injuries comes second after soccer injuries, and lower extremity injury occurs three times more often than upper extremity ³. Moreover, a study conducted in 2010 by Indonesia Ministry of Youth and Sports found 78 of 84 athletes had injuries.
92% of injuries occurred during exercise and most of them were lower extremity.

Researchers and clinicians must pay attention to sport injury prevention and intervention. One of proactive efforts in injury prevention is by performing screening process to detect athlete's injury risk. Lower extremity injury could be affected by a huge biomechanical stress while running and a varied foot posture. Foot morphology is an important factor that determines correlation between ground reaction force and lower extremity rotation axis. A more pronated foot have a bigger mobility, both in lateral medial and vertical planes. A hyperpronated foot allegedly can increase injury risk because it has the potential to cause a great moment of force and instability. Various techniques have been used to assess foot posture, including visual observation, footprint evaluation, foot arch index, to measure heel position in frontal plane and navicular tuberosity position. Foot Posture Index is a quick and convenient visual measurement method to determine normal or abnormal foot posture classification (pronation, supination or normal), based on visually-observed six foot posture criteria.

The research aimed to determine correlation between running activity and pronated foot posture, and to obtain foot posture description of East Java puslatda athletes during running exercise. Foot posture description is one of the information to estimate injury risk, therefore it could increase athletes, coaches and doctors caution against possible injury threats.

Method

The research was conducted at Sport Clinic of Dr. Soetomo General Hospital, Surabaya, Indonesia, using East Java puslatda athletes who followed screening process from April to September 2013. The subjects met inclusion criteria (subjects who practiced a lot of running activities and less running activities) and exclusion criteria (aged less than 18, foot deformities such as syndactyly, polydactyly, terminal phocomelia, sport injuries, subject cannot stand relax and stack weight on both legs e.g. in pain condition), and signed informed consent. The study protocol was approved by the ethics committees of Dr. Soetomo Teaching Hospital (Surabaya, Indonesia).

The research is an observational analytic with cross sectional study design using 75 subjects, consisting of 43 subjects in the group that did a lot of running activity and 32 subjects in the group that did less running activity. The physical examinations included anthropometric and foot examinations, including inspection, palpation, examination of ankle and toe range motion, joint laxity and Foot Posture Index measurement. The process of data analysis was conducted using SPSS 13 software (SPSS, Inc., Chicago, IL). The obtained data were then statistically analyzed using logistic regression.

Results

Table 1. Logistic regression analysis on pronated posture-related factors (bivariate)

<table>
<thead>
<tr>
<th>Variabel</th>
<th>B</th>
<th>Harga p</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>0,782</td>
<td>0,126</td>
<td>0,043</td>
</tr>
<tr>
<td>Age</td>
<td>0,036</td>
<td>0,532</td>
<td>0,007</td>
</tr>
<tr>
<td>BMI</td>
<td>0,069</td>
<td>0,931</td>
<td>0,00</td>
</tr>
<tr>
<td>Exercise duration</td>
<td>0,095</td>
<td>0,149</td>
<td>0,039</td>
</tr>
<tr>
<td>Running activity</td>
<td>0,507</td>
<td>0,293</td>
<td>0,20</td>
</tr>
</tbody>
</table>

R² was obtained from Nagelkerke
Table 2. Logistic regression analysis on pronated posture-related factors (multivariate)

<table>
<thead>
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<th>Variables</th>
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<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
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<td>0.032</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.039</td>
<td>0.696</td>
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<tr>
<td>BMI</td>
<td>0.089</td>
<td>0.923</td>
<td>0.132</td>
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<tr>
<td>Exercise duration</td>
<td>0.080</td>
<td>0.237</td>
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<tr>
<td>Running activity</td>
<td>1.048</td>
<td>0.062</td>
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</table>

R² was obtained from Nagelkerke.

Table 3. Logistic regression analysis on pronated posture-related factors in male subjects

<table>
<thead>
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<th>Variables</th>
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<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise duration</td>
<td>0.508</td>
<td>0.008*</td>
<td>0.275</td>
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<tr>
<td>Age</td>
<td>-0.328</td>
<td>0.024*</td>
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</tr>
<tr>
<td>BMI</td>
<td>-0.764</td>
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<tr>
<td>Running activity</td>
<td>0.304</td>
<td>0.658</td>
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</tr>
</tbody>
</table>

R² was obtained from Nagelkerke.

Table 4. Logistic regression analysis on pronated posture-related factors in female subjects

<table>
<thead>
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<th>Variables</th>
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<th>p</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exercise duration</td>
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<td>0.512</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.209</td>
<td>0.230</td>
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<tr>
<td>BMI</td>
<td>19.596</td>
<td>1.0</td>
<td>0.363</td>
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<tr>
<td>Running activity</td>
<td>22.285</td>
<td>0.99</td>
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</table>

R² was obtained from Nagelkerke.

The study had a sample of 75 subjects, consisting of 43 subjects in the sample group that did a lot of running activity and 32 subjects in the group that did less running activity. The average subjects’ age in the group that did a lot of running activity was 22.8 with age range between 18.0-33.9, while the average subjects’ age in the group that did less running activity was 23.5 with age range between 18.0-31.2.

The measurement of left foot posture obtained 47 normal postures (62.7%), 27 pronated postures (36%) and 1 hyperpronated posture (1.3%). In the squash group, there were 3 normal postures (37.5%) and 5 pronated postures (62.5%). 5 normal postures (55.6%) and 4
pronated postures (44.4%) were found in the martial arts group, while there were 12 normal postures (60%), 7 pronated postures (35%) and 1 hyperpronated posture (5.0%) in the sepaktakraw group. In the karate group, there were 3 normal postures (100%). Moreover, there were 7 normal postures (100%) in the athletic group, 8 normal (61.5%) and 5 pronated postures (38.5%) in the softball group, 4 normal (40%) and 6 pronated postures (60%) in the baseball group and 5 normal postures (100%) in the table tennis group (Table 5.4). There were differences between left and right foot postures in the group that did a lot of running activity, while there was no difference between left and right foot postures in the group that did less running activity. The differences were found in 8 subjects, consisting of 2 squash subjects, 1 martial arts subject, 3 sepaktakraw subjects and 2 karate subjects.

Moreover, the study found 1 normal posture (2.3%) and 1 pronated posture (3.2%) in the group with less BMI, 39 normal postures (88.6%) and 27 pronated postures (87.1%) in the group with normal BMI, as well as 7 normal postures (9.3%) and 3 pronated postures (9.7%) in the group with excess BMI.

Correlation between Running Activity and Pronated Foot Posture

The results of multivariate logistic regression analysis found that running activity, age, body mass index and exercise duration variables did not show significant results. Meanwhile, sex variable showed significant results with \( p < 0.05 \) (0.032) (Table 5.9 and 5.10).

The results of multivariate logistic regression analysis in the male sample group showed that age and exercise duration variables had significant results (*), while BMI and running variables did not show significant results (Table 5.11).

Moreover, the results of multivariate logistic regression analysis in the female sample group showed that BMI, running, age and exercise duration variables did not have significant results (Table 5.12).

Discussion

The research found no significant difference between sex and exercise duration in both sample groups. On the other hand, there was a significant difference between sex \( (p = 0.001) \) and body mass index \( (p = 0.049) \). The number of subjects mainly affected these findings since there were 28 males and 4 females in the group that run less than 20 km per week, while there were 20 males and 23 females in the group that run more than 20 km per week. Moreover, there were 5 subjects with excess BMI in the group that run less than 20 km week, while there were 2 subjects with excess BMI in the group that run more than 20 km week.

Running Activity

Running provides external and internal forces in the run musculoskeletal system. External forces include resistance of air, gravitation and GFR, while internal forces are given by muscles and tendons in the joints. Bone and ligament structures should hold the GFR three to four times the normal weight while running. The present study found two running distances performed by the athletes of different sports. The running distance data were classified into group with running distance more than 20 km per week and group with distance less than 20 km per week. The group with a lot of running activity consisted of squash, martial arts, sepaktakraw, karate and athletics with an average exercise duration of 9 years. Meanwhile, the group with less running activity consisted of softball, baseball and table tennis with an average exercise duration of 8.5 years. The body forces during running activity correlated with foot pronation’s magnitude and speed. Foot pronation is a protective mechanism during running activity that could spread the force for a longer time compared to the condition without pronation. The use of shoes and orthosis during exercise could maximize performance and minimize trauma risk. All subjects in the present study used running shoes during running exercise.

Foot Posture Measurement Results

Foot posture was measured using Foot Posture Index with 6 measurement criteria, including talar head palpation, supra and infra lateral malleolar curvature, calcaneal frontal plane position, prominence in the region of the talonavicular joint, congruence of the medial longitudinal arch and abduction/adduction of the forefoot and the rearfoot.

Running activity

The pronated foot posture measurement results showed a higher percentage in the group with running activity more than 20 km per week. There were differences between right and left foot postures in the
group with a lot of running activity. These findings allegedly correlated with extremity dominance.

**Age**

Degenerative process, incomplete trauma healing and large mechanical loads allegedly become the cause of tendon rupture. The flat foot deformity is often associated with posterior tibial tendon dysfunction. Posterior tibial tendon dysfunction does not always occur due to trauma, but it commonly occurs due to degenerative process. The condition is more prevalent in females aged 50-60. The present study found no correlation between pronated foot posture and age. This finding was allegedly affected by the subject’s age range (18-33.9 years old).

**Sports**

The study found that pronated foot posture was more prevalent in squash, karate and baseball. This might be due to a correlation between athlete’s foot morphology and sports. Some data suggest that specific exercise and repetitive movements in certain sports can affect foot arch index 1. Further studies are necessary in order to determine correlation between pronated foot posture and sports.

**Sex**

The study found that pronated foot posture was commonly found in the male subjects (23;74.2%) compared to females (8;25.8%). The statistical results showed no significant correlation between sex and foot posture in bivariate logistic regression test, with p=0.126. These findings were consistent with a study conducted by Rodriguez et al. (2013) that found no difference between male and female foot posture index. Multivariate logistic regression test found a significant correlation between sex and foot posture, with p=0.032. This finding indicated that age variable was a confounding factor.

**Body Mass Index**

Body weight is transmitted into foot through ankle. When standing and silent, about half the weight rests on the heel. In this condition, talar head will shift to medial as much as 2-6 mm, while lateral turbecle from calcaneus will shift to media as much as 2-4 mm. Rearfoot will be more adductive-pronation, while forefoot will be more flexyabductive-supination 9. Increased mass with an addition of 20% body mass will result in static and dynamic plantar pressure, but it is not significantly change foot structure. Increased long-term body mass index associated with obesity can be seen in the horizontal medial longitudinal arch on the legs, confirmed by increased foot contact areas on the floor. This change is unclear whether permanent or reversible 10. The present study found no significant correlation between body mass index and foot posture, with p=0.931. This might be due to no subject classified as obesity.

**Correlation between Running Activity and Foot Posture**

In his study conducted in 1323 professional athletes, found an increased flat foot and splay foot risks in the endurance runner group 2. After a 20-kilometer run, the force distributed on the legs increased on the forefoot, middle foot and medial heel, while decreased force occurs in the lateral toe. The foot posture study which was measured immediately after runners run at a speed of 3.3 m/s for 60 minutes showed foot posture change. 10 of 30 participants showed a more pronated posture after running, and there was an increased pressure in the plantar. This finding showed an excess force in the medial heel.

The present study found no significant correlation between running distance and pronated foot posture, with p = 0.293. The research hypothesis was not proven. This might be due to a distant foot posture measurement after the subjects performed running activity. This finding was consistent with a study conducted by Karagounis et al. that examined correlation between increased plantar pressures after running. They found that this increment would return again as before after 24 hours. Bisiaux and Moretto in their study found increased plantar pressures at 30 minutes after exercise did not return at 30 minutes after break. This plantar pressure change is allegedly correlated with muscle fatigue.

**Conclusion**

The research found no correlation between running activity more than 20 km per week and pronated foot posture. A higher percentage of pronated foot posture was found in the group that run more than 20 km per week, but it was not statistically significant.

**Conflict of Interest:** There is no conflict interest

**Source of Funding:** This study is self-funded
Indian Journal of Forensic Medicine & Toxicology, April-June 2020, Vol. 14, No. 2

**Funding:** Self-funding

**Ethical Clearance:** Taken from Ethic Committee of Faculty of Medicine, Universitas Airlangga

**Conflict of Interest:** The authors declare that there is no conflict of interest in this study.

**References**


Morphometry Study on Thoracic Vertebrae Pedicle withComputed Tomography Scan in Population of Surabaya,Indonesia

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Abstract
The fixation technique with pedicle screw is widely used for stabilization as well as deformity correction. However, data on vertebral morphometry have not been obtained. Most previous studies were conducted on the Caucasian race and slightly in the Asian race considering that there was a significant difference in pedicle morphometry of vertebrae between races and age. The objective to examine the morphometry of the thoracic vertebral pedicle in young adult population of Surabaya, comparing with the pedicle morphometry in the overseas population. The method we collected patients’ data of thorax CT Scan in Dr. Soetomo Teaching Hospital and Airlangga Hospital Surabaya. Patients with spinal deformities including congenital abnormalities, trauma, infection, and tumors were excluded. The variables to be measured were pedicle diameter (Pedicle width), pedicle depth to anterior cortex corpus vertebrae (Cord Length), and transverse pedicle angle. The result CT scan data collected was 123 consisting of 56 males and 67 females with an average age of 18.1 years old. The average pedicle width, cord length, and transverse pedicle angle in Surabaya population differed significantly from the Caucasian population of the previous study. Conclusion there were no significant differences in transverse pedicle angle between males and females. There was no significant difference in morphometric data compared to the Asian population.

Keywords: Thoracic Vertebrae, Pedicle Screw, Pedicle Morphometry, Surabaya Population

Introduction
The use of pedicle screw as a fixation tool on spine surgery has become popular all over the world. This procedure is the only system that can provide fixation on three vertebral columns and restrain movement in all dimensions¹. Accurate and comprehensive antopometric data for the spine (vertebrae) is not available at this time. Information about the dimensions of the vertebrae is essential for the development and design of rational spinal implants such as pedicle screw. Previous studies have measured this with X-ray modalities, and computed tomography (CT) scan². Several studies also performed analysis on cadaveric specimens³.

The thoracic vertebrae have unique characteristics and these characteristics are associated with potential complications of injury to the neural structure. The magnitude of this potential complication that causes the use of pedicle screw on the thoracic vertebrae is very limited. The pedicle diameter of the thoracic vertebrae is sometimes smaller than the available pedicle screw diameter⁴. The lateral aspect of the dural sac is directly adjacent and attached to the medial wall of the pedicle. Therefore, misalignment of screw mounting in the medial direction may cause injury to the dural sac and spinal cord⁵, and lateral errors can cause injury to the blood vessels⁶.
A study in Asian populations and using CT scans showed that the data is not applicable for the Caucasian population. Recently, the development of digital imaging and computer usage has led us to a new generation of X-ray imaging that enables the manipulation and improvement of the resulting image quality, and allows precision measurements in different areas of the vertebrae. These data can produce basic anthropometric models for the study of spinal implant design.

Most of the anatomical studies of vertebrae pedicle morphology were conducted in the Caucasian population, and a small percentage in the Asian population. Several studies reported no statistically significant difference between the data examined on CT scans and on cadavers. Previous studies have also shown significant differences in pedicle size between human races. Therefore, we aimed to examine the morphometry of the thoracic vertebral pedicle in young adult population of Surabaya, comparing with the pedicle morphometry in the overseas population.

Method

The subjects of this study were patients of Dr. Soetomo Teaching Hospital and Universitas Airlangga Hospital Surabaya, Indonesia that fulfilled the criteria of inclusion and exclusion. The inclusion criteria of the subjects included patients aged 16-25 years old and patients underwent thoracic CT scan with all indications. The exclusion criteria included patients with spinal deformities including congenital abnormalities, trauma, infections, and tumors. Subjects who were willing to participate to the research filled out the informed consent sheet.

This study used cross-sectional observational design that described the average morphometry of young adult vertebrae pedicle in population of Surabaya, Indonesia and compared morphometry of vertebrae pedicle among young adult population in Surabaya, Indonesia and overseas population whose data obtained from previous study. The process of determining the number of subjects included identification according to the inclusion and exclusion criteria in which the subjects obtained 123 patients consisting of 56 male patients and 67 female patients. The number of thoracic vertebrae examined was 1476 pieces consisting of 672 pieces of male vertebrae and 804 pieces of female vertebrae.

The research procedure was performed based on the results of 16 slices of thorax CT Scans (Siemens Ltd, Berlin and Munich, Germany) on the subjects. The CT-Scan data was a opened with the application of Osirix version 6.5. Furthermore, by using the application, the file of CT-Scan can be opened, re-formatted, and measured the variables needed. Measurements were performed on the vertebral pedicle T1 to T12. The variables to be measured were pedicle diameter (mm), pedicle to anterior cortex corpus vertebrae (mm), and pedicle transversal angle(°). The pedicle width was determined by measuring the shortest distance between the pedicle walls. The transverse angle pedicle was determined by measuring the angle formed between the central axis of the pedicle and the perpendicular line from the pedicle width line passing through the pedicle. Chord length was measured from the posterior and anterior cortical ends of a line perpendicular to the pedicle width used in the transverse pedicle angle measurement. All measurements used axial piece of CT scan.

Measurable structures in the vertebrae included (1) chord length of the pedicle, measured from the posterior cortex (entry point) pedicle to the anterior cortex of the vertebrae in line with the pedicle axis, (2) length of the pedicle, measured from the pedicle posterior cortex to the posterior longitudinal ligament along the pedicle axis, (3) transverse pedicle diameter, measured at isthmus horizontal orientation, (4) the pedicle insertion angle, measured from the midline towards the pedicle mid-axis, (5) the height of the pedicle, measured in the vertical orientation isthmus, (6) the length or width of the vertebral corpus, measured from the posterior to the anterior longitudinal ligaments on the mid-sagittal pieces (measurements were made on both end plates, and the average value was calculated), (7) the pedicle offset, calculated from the spinous processus to the medial and lateral walls of the pedicle, and from the inferior facet to the superior extent of the outer wall of the pedicle, and (8) the pedicle’s entry point (central axis), measured at the confluence between the transversus processus and the nearest facet joint.

After all variables were obtained, the mean and standard deviation measurements were performed on each variable. The measurement were differentiated between males and females at each vertebral level to look for significant differences. Due to the absence of gender discrimination in previous studies defined as comparative studies, measurements of mean and standard deviations of all samples were divided regardless of
gender. Furthermore, these data were compared with previous research.

**Results**

In both males and females, the average pedicle width was highest at T12 level with a magnitude of 9.05 mm and 7.63 mm respectively. The diameter of the pedicle gradually decreased from T1 until it reached the smallest diameter in T4, and gradually enlarged again. The narrowest diameter was at T4 (male: 4.75 mm; female: 3.98 mm). The diameter of the vertebra pedicle was significantly larger in males compared with females at all levels.

Pedicle chord length was significantly shorter in females than in males at all vertebral levels. The pedicle chord length ranged from 27.28 mm to 50.98 mm in males and from 25.88 mm to 43.84 mm in females. The longest pedicle chord length was at 50.98 mm and the shortest was at 25.88 mm in males and females. The length of the pedicle chord increased from T1 to T9, and shortened from T10 to T12. Compared with previous research data, the trend was found similar.

The level with the widest transverse pedicle angle was T1 (36.29°) in males and also T1 (35.71°) in females. The transversal pedicle angle gradually narrowed to its level from T1 to T12, and the narrowest at T12 (male: -4.08°; female: -6.26°). No significant difference was found between the transverse pedicle angle of male and female subjects. Compared with other studies, no significant difference was found in the transverse pedicle angle, but had similar trends in the pattern.

**Tabel 1: Comparison of pedicle width, cord length, and transverse pedicle angle in males and females**

<table>
<thead>
<tr>
<th>level</th>
<th>pedicle width</th>
<th>cord length</th>
<th>transverse pedicle angle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male Female p</td>
<td>Male Female p</td>
<td>Male Female p</td>
</tr>
<tr>
<td>T1</td>
<td>7.25±0.68 6.68±0.69 0.000</td>
<td>35.50±2.19 32.50±1.96 0.000</td>
<td>31.23±2.52 30.65±2.54 0.208</td>
</tr>
<tr>
<td>T2</td>
<td>7.04±0.73 5.60±0.92 0.000</td>
<td>36.14±2.50 32.87±2.12 0.000</td>
<td>20.78±2.31 20.90±2.65 0.791</td>
</tr>
<tr>
<td>T3</td>
<td>5.38±0.84 4.33±0.83 0.000</td>
<td>35.97±3.16 32.82±2.65 0.000</td>
<td>12.97±2.42 12.90±2.56 0.877</td>
</tr>
<tr>
<td>T4</td>
<td>4.75±0.53 3.99±0.74 0.000</td>
<td>35.07±2.75 33.36±3.11 0.000</td>
<td>10.29±2.17 10.13±2.11 0.680</td>
</tr>
<tr>
<td>T5</td>
<td>4.83±0.67 4.08±0.87 0.000</td>
<td>37.28±2.90 33.70±2.73 0.000</td>
<td>8.52±2.03 7.82±2.24 0.074</td>
</tr>
<tr>
<td>T6</td>
<td>5.11±0.94 4.12±0.78 0.000</td>
<td>38.11±2.44 34.74±2.47 0.000</td>
<td>6.95±1.93 6.71±2.25 0.531</td>
</tr>
<tr>
<td>T7</td>
<td>5.15±0.86 4.33±0.85 0.000</td>
<td>40.84±2.43 35.95±2.86 0.000</td>
<td>5.34±1.94 5.37±2.68 0.944</td>
</tr>
<tr>
<td>T8</td>
<td>5.67±0.82 4.53±0.81 0.000</td>
<td>41.04±2.76 36.64±2.35 0.000</td>
<td>3.88±2.50 4.43±2.54 0.230</td>
</tr>
<tr>
<td>T9</td>
<td>5.87±0.73 4.77±0.89 0.000</td>
<td>41.15±3.54 37.13±2.83 0.000</td>
<td>2.51±2.08 3.50±2.28 0.013</td>
</tr>
<tr>
<td>T10</td>
<td>6.79±1.13 5.82±1.16 0.000</td>
<td>40.79±3.88 35.80±2.88 0.000</td>
<td>1.25±1.78 1.51±2.34 0.496</td>
</tr>
<tr>
<td>T11</td>
<td>8.28±1.13 7.32±1.46 0.000</td>
<td>40.30±3.14 35.45±2.46 0.000</td>
<td>-0.71±2.04 -0.28±1.80 0.216</td>
</tr>
<tr>
<td>T12</td>
<td>9.05±1.69 7.63±1.17 0.000</td>
<td>39.14±3.34 35.13±2.43 0.000</td>
<td>-1.66±1.16 -1.33±1.99 0.275</td>
</tr>
</tbody>
</table>
Discussion

In the caucasian population, previous research examined the morphometry of the thoracic vertebrae pedicle using a CT scan by measuring several parameters. When compared with the results the morphometric size of previous the thoracic vertebral pedicle in the Surabaya population is proved to be significantly smaller. These size differences may be associated with a smaller body size in Asians. Asians have a smaller body mass index, but have a greater percentage of body fat when compared to Caucasians. Therefore, height and body mass index size may provide good predictive value for pedicle size of the vertebrae. Measurement of pedicle size of the vertebrae using a pre-operative CT scan may also provide precision vertical pedicle dimension precision data, and improves the accuracy of pedicle screw mounting on the thoracic vertebrae; thus, the complications can be avoided.

The comparison of mean pedicle width between Kai et al’s study(19) study and some previous studies in Table. It appears that the large pedicle width in the Surabaya population is smaller when compared with the Caucasian population and has similarities with the Asian population. Comparison between Kim et al’s study(5) and the current study chord length pedicle of Surabaya population with other research. It appears that the chord length pedicle in Surabaya population is smaller than the Caucasian population and similar to other Asian populations. The Comparison between Panjabi et al’s study(6) and the current study appears that the large mean transverse pedicle angle in Surabaya population is also smaller than the Caucasian population and similar to other Asian populations.

Researcher performed a comparison between pedicle morphometry in Surabaya population and pedicle morphometry in the Asian population. From these comparisons, it is evident that pedicle morphometry in Surabaya populations does not differ significantly with other Asian populations. There was a comparison of pedicle morphometry between Surabaya population and Caucasian population. From the comparison, the morphometry of pedicle of Caucasian population is significantly greater than Surabaya population.

The Malaysian population compared with our study on the population of Surabaya using two sample T-tests, it was found that both the comparison of pedicle width, cord length, and transverse pedicle angle showed no significant difference. Kai et al. conducted a cross-sectional study of pedicle morphometry in 180 patients of Malaysian Malay race with ages ranging from 18 to 80 years. Kai’s research concludes that the existing pedicle screw system is incompatible with the majority of the Malay population, especially at the midthoracic level. Kai also stated that the smaller pedicle size of the Malay race examined may be related to the Malay race body that is shorter than Caucasian races.

Conclusion

CT scan evaluation of the thoracic vertebrae can provide morphometric data which is very useful in determining the length and length of the pedicle screw, and determining the direction of the pedicle screw on transpedicle spinal surgery. The size of the pedicle’s morphometry in humans varies from one to another. In this study, it appears that the morphometry of the human pedicle differs from one population to another. Smaller thoracic vertebrae pedicle dimensions in Surabaya population compared with Caucasian population can be associated with smaller body size in Asian populations. Many factors that cause this difference are: race, weight, height, and body mass index. Therefore, in carrying out the transpedicle instrumentation action, it is necessary to consider these factors and, if possible, CT scan can be performed before the surgery to estimate the pedicle dimensions of patients.

Conflict of Interest: There is no conflict of interest

Source of Funding: This study is self-funded

Ethical Clearance: This study was approved by Ethical Comission of Health Research Faculty of Medicine University of Airlangga

References

4. Kothe R, O’Holleran JD, Liu W, Panjabi MM. Internal architecture of the thoracic pedicle:


Awareness about Whole Slide Imaging and Digital Pathology among Pathologists - Cross Sectional Survey

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¹Postgraduate, ²Professor and Head of the department, ³Professor, ⁴Reader, ⁵Senior Lecturer, Dept. of Oral Pathology, Saveetha Dental College & Hospitals, Saveetha University

Abstract

Aim: To assess awareness of pathologists on whole slide imaging and digital pathology

Method: An online, structured questionnaire was sent to pathologists of various dental and medical colleges in Chennai. The data was collected using Microsoft excel version 2011 and statistically analyzed using SPSS software.

Results: 28.6% of pathologists were only aware of the fact that whole slide imaging can perform a live scanning of digital images. 81% said the magnification produced by WSI were greater than conventional microscopy. 52.4% agreed on the time taken to perform the whole slide scanning to be one minute. 85.7% were convinced that diagnostic difficulties could be best consulted through the digital slides. 52.4% agreed integrated automated image analysis increased the diagnostic efficacy of WSI in routine histopathology. 62.5% of them felt that the high cost of the equipment and infrastructure was the major impeding factor in usage of WSI.

Conclusion: Whole slide imaging and digital pathology is a promising future of pathology as it can significantly reduce the workload and enhance the diagnostic efficiency in various aspects. There was a reasonable awareness among the pathologists. Until hospitals collaborate and take initiative to bring whole slide imaging into practice, it’s difficult to make the best of its advantages.

Key words: Pathologists, Digital pathology, virtual slides, whole slide imaging

Introduction

As digitalization embracing through any and every known field, Pathology is no exception. Time and again, many digital technologies have been introduced into pathology starting from digital photomicrographs (1900s) to Telepathology (1980s) to Digital pathology involving whole slide imaging (2000s). Whole slide imaging refers to scanning and storage of entire histopathological glass slide into set of high-resolution digital files that can be interpreted using image viewers on computer workstations.

The complete digitalization of slides has the potential to transform practice of diagnostic pathology – safety, quality and efficiency of histopathological diagnosis. Despite the promise of digital pathology to offer huge benefits, its uptake in clinical practice has been slow. One of the main reasons was considered to be the unfamiliarity with the viewing software and perceived inefficiency of digital diagnosis. Clearly cannot change instantaneously.

The present study forms the first step in bridging the gap between the pathologists and digitalization. It aims to assess the awareness level of Pathologists on whole slide imaging and its advantages as future of pathology.

Materials and Method

The study was designed to be a cross-sectional survey since it aimed to evaluate the awareness of pathologist on given point of time. An online questionnaire was designed to assess the knowledge, attitude of pathologists towards the whole slide imaging technique and digital pathology and its future perspectives.
The questionnaire was prepared using Google forms and distributed online to general pathologists and oral pathologists of various dental and medical colleges in Chennai. The pathology postgraduates and lectures of pathology department were randomly selected and invited to participate in the study. The questionnaire had sections, which gave a brief introduction about scope of the study, email address was collected and the questions with multiple choices had been provided. Explanations were given if the any of the participants could not understand the items.

The data was collected and analyzed through the Google forms using Microsoft excel version 2011.

Results

Forty-two completed responses were collected online. Four out of ten questions evaluated the awareness on the technical aspects of WSI and DP. Three questions were on the applications of WSI and DP. Three were questioned to assess the knowledge on practical aspects of WSI and DP.

66.7% pathologists were aware Whole slide imaging utilizes the advantages of digital images and live cameras. 81% said the magnification produced by WSI were greater than conventional microscopy. 52.4% felt the whole slide scanning can be done with one minute. 47.6% pathologists opined that high speed scanners are major indispensible need to put DP and WSI to daily use while 35.4%f elt the need to be high resolution display monitors. The major hindrance in adopting widespread practice of WSI and DP were felt to be the High cost involved by 62.5% pathologists and technical factors by 27.5% pathologists. 85.7% pathologists were convinced that digital slides achieved faster consultations at its best. The promising role of DP and WSI in forming next-generation education were felt to be the scalable nature of digital file & availability to numerous students at a time by 33.3% pathologists, due to the incorporated annotations, videos, sound clips by 33.3 % and due to whole 3D picture of the specimen provided by DP by 33.3% pathologists. 59.5% felt that integration o WSI to pathology reports while 36.6% felt it was accessibility of WSI on iPads and PDA.

Discussion

Our study was based on data from online questionnaire and rate of return of these questionnaire reflect maximum amount of interest in whole slide imaging and digital pathology. Research into whole slide imaging ad digital pathology has been in progress for a decade. [1,2] All have inherent problems yet interest continues to increase worldwide with a growing consensus regarding acceptable applications, [3,4] particularly those related to teaching and quality assurance.

The practical experience of Whole slide imaging and Digital pathology are uncommon among histopathologists in India. Consequently there is an understandable skepticism with regard to technology and its ability to enhance conventional method of practice. Capital costs of installing DP and WSI are really high (particularly the image scanners). [5]

Many of the fears regarding DP and WSI have arisen through unfamiliarity with technology and can be overcome by onsite technical assistance combined with protocol driven referral pathways. [5] 66.7% of the pathologists perceived the virtual pathology to be a set of digital images only, although it involves recording digital images of the pathology slides with live scanning. This predicts the bird’s view, not a detailed understanding of the technology.

Although several reports of enhanced teaching experiences had been witnessed through digitalization in western countries, [6,7,8] less cumbersome software along with adequate training of teaching faculties in them, and more easier identification of structures with clarity can help increase the level of acceptance in India. 85.7% of pathologists felt the unanimous solution on difficult diagnostic cases would be through digital consultations with their distant colleagues. Rapid remote initial diagnosis is possible as is consensus diagnosis for problematic cases. [9] The human resources can be maximized by remote participation.

52.5% felt the automated analysis of histopathology slides could help reduce their work burden give an accurate diagnosis in a quick time interval especially when it comes quantitative measurement of increased mitosis and other prognostic factors in cancers. [1,10] 52.5% of the pathologists the scanning speed to be under 1 minute. The latest technology scanners offer to complete the whole slide scanning in just <50 seconds. Al-Janabi et al has documented a success rate of 82% for primary reporting with digital slides. [11] Snead et al had
reported 97.7% complete concordance between glass and digital slide diagnosis. [12]

47.6% of the pathologists believed the high speed “slide scanners” are the major requirement to keep digital pathology in day-to-day diagnosis. These scanners provide the high-resolution images of the entire tissue section but at an outrageous cost. This can hinder its application in practice particularly in developing countries with limited resources. Attempts at developing substitutes for these scanners at negligible cost have been showing promising results with their own disadvantages. [13] However the technical advances would help overcome these problems in the future.

**Conclusion**

There is a reasonable level of awareness among pathologists on whole slide imaging and digital pathology. The practical hindrances in adoption of this technique should be overcome to achieve the huge advantage it holds. Innovations on production of low cost, efficient slide scanners are the need of the hour. Government should take initiative in collaboration with private organizations to install Digital set up for whole Slide Images.

**Conflicts of Interest** – Nil

**Source of Funding** – Self-funded

**Ethical Clearance** - Taken from Institutional standard review board (Approval no: SRB /SDMDS16 OMP/08).

**References**

### A: QUESTIONS ON THE TECHNICAL ASPECTS OF WSI AND DP.

<table>
<thead>
<tr>
<th>QUESTION</th>
<th>RESPONSE RATE</th>
<th>ANSWER CATEGORIES</th>
<th>RESPONSE (IN NO.)</th>
<th>RESPONSE (IN %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Principle behind WSI/DP</td>
<td>100%</td>
<td>1. Digital images</td>
<td>28</td>
<td>66.7%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Live video scanning</td>
<td>2</td>
<td>4.7%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. combines Digital images and live scanning</td>
<td>12</td>
<td>28.6%</td>
</tr>
<tr>
<td>Magnification produced by digital slides</td>
<td>100%</td>
<td>1. Equal to conventional slides</td>
<td>7</td>
<td>16.7%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Greater than conventional slides</td>
<td>34</td>
<td>81%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. Lesser than conventional slides</td>
<td>1</td>
<td>2.3%</td>
</tr>
<tr>
<td>Time taken to perform WSI</td>
<td>100%</td>
<td>1. &lt;1 minute</td>
<td>22</td>
<td>52.4%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. &lt;5 minutes</td>
<td>11</td>
<td>26.2%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. &lt;50 seconds</td>
<td>9</td>
<td>21.4%</td>
</tr>
<tr>
<td>Full size of WSI</td>
<td>100%</td>
<td>1. Megabytes</td>
<td>12</td>
<td>28.6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Kilobytes</td>
<td>5</td>
<td>11.9%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. Few megabytes to several gigabytes</td>
<td>25</td>
<td>59.5%</td>
</tr>
</tbody>
</table>

### 1B: QUESTIONS ON THE APPLICATIONS OF WSI AND DP.

<table>
<thead>
<tr>
<th>QUESTION</th>
<th>RESPONSE RATE</th>
<th>ANSWER CATEGORIES</th>
<th>RESPONSE (IN NO.)</th>
<th>RESPONSE (IN %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digital slides for next generation teaching</td>
<td>100%</td>
<td>1. Functions as scalable microscope for unlimited students</td>
<td>14</td>
<td>33.3%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Possibility of annotations, videos &amp; sound clips</td>
<td>14</td>
<td>33.3%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. Provides 3-D stacks of sections</td>
<td>14</td>
<td>33.3%</td>
</tr>
<tr>
<td>Best consultations for difficult/ rare cases</td>
<td>100%</td>
<td>1. By regular mail</td>
<td>4</td>
<td>9.5%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. By shipping slides</td>
<td>2</td>
<td>4.8%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. By digital slides</td>
<td>36</td>
<td>85.7%</td>
</tr>
<tr>
<td>Diagnostic efficacy of DP in histopathology</td>
<td>97.6%</td>
<td>1. Automated image analysis</td>
<td>22</td>
<td>52.4%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Constant quality of images</td>
<td>11</td>
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<td></td>
<td></td>
<td>3. Integrating WSI to pathology report</td>
<td>9</td>
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### 1C: QUESTIONS ON PRACTICE OF WSI AND DP

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<th>Answer Categories</th>
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<td>1. High speed scanners</td>
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<td>2. High cost involved</td>
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<td>3. Integration of WSI with laboratory workflow</td>
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</table>
Alteration in Physicochemical Parameters of Soil Beneath Rabbit Carcass: Consequence of Carcass Decomposition

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Abstract

Forensic taphonomy involves the use of decomposition to estimate post-mortem interval (PMI) or locate cadaveric grave. The process associated with carcass decomposition in outdoor setting. Some other methods are available to determine the accurate post mortem interval (PMI). Instead of these methods, a soil-based approach one of the best methods to determine post mortem interval. As a consequence, we investigated the physical characteristics of the carcass, soil pH, soil moisture content, electrical conductivity and the concentration of total carbon, total nitrogen and soil-extractable phosphorus in soil beneath rabbit (Oryctolagus cuniculus L.) carcass which was placed on soil surface up to skeletal stage of decomposition. The significant increases were observed in concentration of soil pH, moisture content, soil-extractable phosphorus and total carbon.

Keywords: Decomposition stages, Forensic taphonomy, rabbit carcass, physicochemical.

Introduction

Forensic taphonomy is an applied science with clear aims: Use the processes associated with cadaver decomposition to estimate post mortem or post burial interval, determine the manner of death, locate graves and identify the deceased[1,2]. Forensic taphonomy derives these aims from taphonomy, a branch of palaeontology[3]. The majority of these studies have focused on the activity of above ground insects[4,5] and scavengers[6] whereas less attention has been given to the processes that occur in soils associated with cadaver breakdown[7].

The estimation of soil in a demise examination is frequently taken as cooperative proof. This mirrors the customary perspective of criminological science: Soil is a medium that can be characterized by natural substance, and physical properties[8]. Soil has evidential values, it contains mineral, vegetation (both vegetative and as pollen), and animal material (living and dead). The components like fossils, bone and glass are also found in soil, these all are rare and helpful for characterization. Now a day stereomicroscopic observation, scanning electron microscope, x-ray spectrometer, detection of soil colour (before and after heating soil samples) and macroscopic observation are being used for the soil studies. These attributes are generally performed to investigate a serious crime.

Decomposition is a process that commences immediately after death and, depending on environmental conditions, will proceed until skeletonization has occurred[9,10]. The chemistry associated with decomposition and the destruction of soft tissue is complex[11]. During decomposition chemical components are released from body through autolysis and putrefaction[12]. Carcasses are mainly decomposed by microbes and invertebrates[13]. Decomposition results the degradation of carbohydrates, lipids and protein, which will yield carbon-based, phosphorous-based and nitrogen-based products which may be retained in the soil from the body of carcass, therefore, Grave soils were found to have higher levels of total C and total N after burial.

Research model: - A rabbit (Oryctolagus cuniculus L.) carcass weighing about 2.5 kg was used as a research model to study alteration in physico-chemical parameters of soil beneath them. The rabbit carcass was procured
Experimental site: - The course of decomposition of carcass and its impact of soil was studied during March, 2016 at village Miani of District Hoshiarpur, Punjab. The site of experiment was located north side of Dasuya (31°42´15ʺN, 75°35´5ʺE). The climatic condition of experimental site during research period is; the normal annual rainfall of experimental site is 991 mm and average temperature is varying from 24°C to 42°C during experimental period. The soil of experimental site is loamy with pH vary from 7.4 to 8.2. The unwanted vegetation of experimental site was removed. The heavy iron cage was used to cover the carcass to protect them from animal scavengers.

The soil samples were collected with the help of rustproof stainless steel spatula which has no chemical interference with soil. During sampling, approximately 40 g of soil was collected below carcasses and placed into zip lock bags. The collected samples were sieved, placed in sterile vials and transferred to deep freezer (-20°C) for pending analysis[14].

Analysis of Physico-Chemical Characteristics of Soil

Soil pH was calculated with the help of pH meter. One part of soil and five parts of water (1:5) were taken for the analysis[14]. Before the analysis the instrument was calibrated with solutions of different pH i.e. 4.0, 7.0 and 10.0. Electrical conductivity of soil was calculated with the help of digital conductivity meter. Soil and water were taken in 1:5 ratios. Calibration was done with KOH solution[15]. Moisture content of the soil was estimated by heating methods. 5 grams of soil were taken and heated at temperature of 105°C for 24 hours in oven. Weight of pre heated and post heated soil was recorded with the help of weighing balance. Difference between initial and final weight of soil is moisture content of soil.

The extraction of phosphorus from soil has been done according to[16] soil extraction method. The acid-soluble forms of phosphorus were extracted by using combination of Hydrochloric acid and Ammonium fluoride. 1 g of oven dried soil was extracted with diluted of ammonium fluoride (NH₄F) and concentrated hydrochloric acid (HCl). The soil samples were centrifuged at 6000 rpm for 5 minutes. Dispense 0.50 mL of the supernatant plus 2.0 mL colourimetric reagent was added and mix into a test tube to stand for 30 minutes. Soil-extractable phosphorus absorbance was measured with the help of Spectrophotometer at wavelength of 880 nm.

A colourimetric reagent was prepared to dissolved 17.14 g ammonium molybdate [(NH₄)₆MO₇O₂₄.4H₂O] in 200 mL of warm deionised water, 0.392 g potassium antimonyl tartrate (KSB₉C₂H₄O₆) separately in 150 mL deionised water. Place 500 mL deionised water in a 2 L volumetric flask and slowly add 200 mL concentrated sulphuric acid with mixing.

Total carbon and total nitrogen content in soil was analysed by combustion with helium gas and estimated by using Vario EL Cube Elementar CHNS analyser. The temperature of combustion tube was maintained at 1150°C and 850°C for reduction tube. The analysis was conducted at Punjab Agriculture University, Ludhiana (Punjab).

Statistical analysis: - The statistical analysis was conducted by using software SPSS Version 15 to compare variation among parameters in experimental soil and control soil. The variance in parameters was tested by using Levene’s test.

Results

Rabbit carcass took 9 days for complete decomposition. The decomposition was divided into different stages viz. fresh, bloated, active decay, dry decay and skeletal. The soil beneath carcass were taken from three different depths i.e. 0-5 cm top, 6-10 cm middle and 11-15 cm in order to check pH, electrical conductivity and moisture content, but in case of total nitrogen, total carbon, soil-extractable phosphorus upper layer (0-5 cm) of soil were taken into account because most significant changes were occur in top most layer of soil only. This experiment was conducted in the month of March, 2016. The average temperature during experiment was 38±1° C and humidity was 27±1%.

Soil pH

A significant difference (P < 0.05) in pH of control soil and soil beneath carcass was observed (Fig. 1). Soil is become slightly acidic during active decay stage as compare to control soil due to seepage of fluid into the soil.

Electrical conductivity

There was no significant difference (P > 0.05) in electrical conductivity of control soil and experimental soil was observed (Fig. 2). Increase was seen during
active decay stage of decomposition at depth of 5 cm and 10 cm of soil samples as compare to control soil.

**Moisture content**

A significant difference ($P < 0.05$) in moisture content of control soil and experimental soil was observed (Fig. 3). The moisture content of experimental soil was high during bloated, active decay and dry decay stages of decomposition due to seepage of body fluid.

**Soil-extractable phosphorus**

There was a significant difference ($P < 0.05$) in soil-extractable phosphorus of control soil and experimental soil was observed (Fig. 4). This is due to the large number of components like enzymes, phospholipids, proteins; nucleic acids which were store in the form of phosphorus in the body and a decomposing carcass can release high amount of phosphorus in the soil beneath carcass.

**Total carbon**

A significant difference ($P < 0.05$) observed in concentration of total carbon in experimental soil and control soil (Fig. 5). The increase in total carbon concentration of soil was observed during dry decay stage of decomposition. This is due to release of CO$_2$ gas during decomposition.

**Total nitrogen**

There was no significant difference ($P > 0.05$) was observed in total nitrogen content of soil beneath rabbit carcass and control soil (Fig. 6). The increase in total nitrogen concentration observed during dry decay stage of decomposition. However, the nitrogen concentration level returns to basal level by skeletal stage of decomposition.
Fig. 2 Electrical conductivity of control soil and soil beneath rabbit carcass

Fig. 3. Moisture content (%) of control soil and soil beneath rabbit carcass
Fig. 4. Extractable Phosphorus of control soil and soil beneath decomposed rabbit carcass

Fig. 5. Total Carbon (%) content of control soil and soil beneath rabbit carcass
**Discussion**

The present study has significance from forensic point of view and has implication in PMI estimation. In the entire study the biochemical attributes were considered, the alteration in these attributes support the forensic investigation in estimation of time since death as well as place of death by comparing the contents of control soil and soil beneath corpse. The significant increase in concentration of nutrients occurs during bloated stage, active decay stage and dry decay stage of decomposition. Currently forensic entomology is considered as most accurate means to estimate PMI. However, forensic entomology will not able to answer all the questions after emergence of adult blow flies. If proper experimentation occurs then nutrient cycle in soil could be an important tool in cases where bodies remain undiscovered for extended period of time. In current study rabbit carcass was used as research model. Five decomposition stages were observed during whole course of study. Previous decomposition studies provided conflicting results. pH is almost remain same in control soil as well as in experimental soil during fresh stage of decomposition due to negligible seepage of nutrient into the soil. In previous study the increase in pH values was observed during decomposition of carcass\[^{14}\]. In some other decomposition studies by also provide variation in pH level of soil beneath carcasses\[^{17,18}\]. Soil pH may decrease during early stages of decomposition as a result of the fermentative processes carried out by anaerobic bacteria in the gastrointestinal (GI) tract as well as in soil\[^{19}\] and subsequently increase due to carcass proteolysis\[^{18}\]. The reduced level of pH did not increase over time as compare to control soil. The significant changes in soil was seen only in upper layer (0-5 cm) of soil as compare to middle and bottom layers of soil and this is due to the rapid conversion of the Nitrate to Ammonium ion by the microbes. Variations in pH level not able to demonstrate consistent correlation with process of decomposition.

The concentration of soil-extractable phosphorus was significantly increases during the decomposition of carcass. The significant increase detected in soil-extractable phosphorus concentrations beneath a decomposing carcass of pig (*Sus scrofa*) during 100 days of decomposition study of grave soil\[^{14}\]. A significant increase in Phosphorus concentration beneath a decomposing ungulate (*Bos bison*) carcass and given the results that there was a increase in Phosphorus level of soil at carcass site as compare to control soil, upto 3 years post-mortem\[^{20}\]. In addition the large number of components likes enzymes, phospholipids, proteins; nucleic acids are store in the form of phosphorus in the body. After death these all components flow out from the body in form of fluid and mix up with soil. Therefore
a decomposing carcass can release high amount of phosphorus in the soil beneath carcasses.

In present study the total carbon content would increase during bloated and active decay stages of decomposition. Otherwise there was no significant difference between total carbon content of control soil and experimental soil occurred during other stages of decomposition. There was no significant difference of total carbon concentration between control and grave soil was observed beneath decomposed carcass\textsuperscript{[14]}. The total carbon concentration in soil was due to carbon dioxide gas could release from the body of carcass and mixed in soil or lost in environment.

A significant increase in total nitrogen content has been reported in present study which was similar with the previous study which was conducted on gravesoil\textsuperscript{[14]}. The significant increase was observed in total nitrogen concentration of soil beneath decomposing carcass\textsuperscript{[21]}. There is an either organic or inorganic form, i.e. NO\textsubscript{3}\textsuperscript{-N} and NH\textsubscript{4}\textsuperscript{+N} of nitrogen which helps to increase the level of nitrogen. A decomposing carcass is a rich source of nitrogen.

**Conclusion**

The present study satisfactorily demonstrated that decomposition of carcass influxes a significant amount of nutrients into the soil beneath carcass. There is a significant variation in some physico-chemical parameters of soil beneath carcass as compare to control soil viz. Soil pH, moisture content, soil-extractable phosphorus and total carbon due to influx of nutrients into the soil. There is also variation among parameters according to stages of decomposition, which assists in PMI estimation in case where decomposed remains scavenged or displaced from its original position.

The results of this study are only applicable to the local soil environment, region and area from where soil samples were taken and cannot be extrapolated to other soil types or conditions. Hence, future studies will also investigate the nutrient influx from decomposing remains in a range of soil types to determine whether those patterns observed in this study are reproducible in different soil conditions.

We conclude that the effect of a rabbit carcass on the nutrient concentrations of soil is evident in this part of world. We believe that the decomposition of human remains in this environment would also increase nutrients level into the soil. This information has the potential to assist investigations in confirming the presence of decomposed remains where remains have been scavenged or dragged from their original site, or in shallow grave sites where relocation of the remains has occurred post-mortem. In spite not include in the present study there is a suggestion of utilizing soil microorganisms to estimate the PMI. Soil microbiology along with carcass microbiology plays important role in flawless evaluation of PMI.

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**Conflict of Interest:** Nil.

**Ethical Clearance:** Not required.

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Knowledge and Awareness of Medical Students about Injuries in Forensic Perspective

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Abstract

Introduction: Injury is any harm caused illegally to a person, which may be caused by any mechanical force which results in a break in natural continuity of any tissue. Each and every injury as its own medico-legal importance which is the key in interpretation of specific injury. Thus every medical student must be clear about this, which helps in concluding different injuries as every medical practitioner has to deal with injuries throughout their life. This can be dealt only when there is proper knowledge and awareness about different injuries in medical field.

Objective: This study was done to study knowledge and awareness of medical students about injuries in forensic perspective.

Materials and Method: It is a cross-sectional study done at Saveetha Medical College, Chennai, Tamil Nadu. Students of 2nd, 3rd, and 4th year M.B.B.S participated after giving informed consent. 115 students participated in the study. Questionnaire containing 20 questions were distributed and responses were obtained and analyzed.

Results: Out of 115 students, 95% of the students had basic knowledge on injuries. Though awareness was present, there was minimal lack in certain areas such as knowledge on contusions, grievous hurt and differentiating cut and chop wounds.

Conclusion: The knowledge and awareness on various injuries is very important for every medical student and practitioner for recognizing and differentiating it. So students should gain more knowledge and awareness on injuries.

Key Words: Injury, Medical students, Knowledge and awareness, Forensic perspective.

Introduction

Legal definition of injury: Section 44 IPC defines injury as “any harm what so ever illegally caused to any person in body, mind, reputation or property.” Medically, a wound or injury is a break of natural continuity of any of the tissues of living body. Injuries can be classified medically, legally and medico legally.

An injury is always caused by some mechanical force, in which there may be a counterforce given by the body or rigid stationary object when there is movement of person or combination of both. Thus the energy produced is transferred into a person’s tissue. A tissue is a complex structure thus the energy is not transferred uniformly which causes compression or traction or combination of both. Rigid structures such as bones, resist deformation, but if the limit exceeds fracture occurs. Mechanical force to soft tissues alters their shape, which results in cohesion between the tissue cells, connective and vascular tissue frameworks and capsules of organs. Soft tissues rupture when they are stretched beyond the limits of their tensile strength. [8]

Mainly, injuries are due to mechanical or thermal or electrical or chemical etc. Each and every form of injury as its own medico-legal importance. Injuries have acquired least importance among doctors, despite of being one of the leading causes for complications and death. Many things can be done to gain understanding on specific injuries and examine. Any injury can give clues for its occurrence by proper examination, for example,
bite marks, nail tissue scrapings. So any medical person can be effective in this, but can be successful only by gaining knowledge and getting more exposure. This is the duty of every medical practitioner. This can be done only when there is good understanding on injuries and their medico-legal importance during the course of study. Many aspects like, cause of injury, time since it happened, manner of injury, site of impact, direction of force applied, position of the victim and assailant, place of injury, motive of the offence and many factors can be concluded only with proper knowledge on injuries. Even ante mortem and postmortem injuries can be differentiated. Every doctor should know that irrespective of their speciality, they are going to deal with medico-legal aspects of injuries comes throughout their life. Thus the key part of any medical practitioner is to assess appropriately, document clearly and interpret the injuries which were occurred. Medical practitioners are constantly exposed to examination of victims and handle medico-legal aspects of injuries. They are often subjected to court of law, so if they lack knowledge it will lead to negative conclusions. The interpretation and assessment of different injuries can be done only when there is good awareness and knowledge about injuries among medical practitioners. Mechanical injuries have great part in interpretation and assessment and also important in medico-legal aspects, so they were included mainly in this study were knowledge and awareness of different types of injuries among medical students was checked.

**Materials and Method**

This cross-sectional retrospective study was conducted in Saveetha medical college and hospital, Chennai. Undergraduate medical students underwent this study. 115 students participated in the study. Students of 2nd year, 3rd year and 4th year MBBS were included in this study Students of 1st year was excluded as they lacked knowledge on forensic medicine. Study period was from 21st January 2019 to 30th March 2019. This study was done with the help of specially prepared questionnaires for which the validity was checked. It had about 20 questions and they were asked to tick their choice of option. The questionnaire consisted of knowledge on various injuries regarding forensic science such as abrasions, lacerations, contusions, chop and cut wounds, hesitational cuts, grievous hurt and their importance medico legally. The participants were assured that participation was voluntary and confidentiality would be maintained. The Approval of the ethical committee was done before starting. The questionnaires were given to students after explaining the importance of the study. Informed consent was obtained from students who participated and the responses were collected. 120 questionnaires were distributed out of which 5 students didn’t sign the informed consent; so, it was excluded. The data was then entered in MS Excel and was analyzed and percentages were obtained using SPSS software.

**Results**

Total 115 students participated in the study. In general 95% of the medical students had basic knowledge and awareness about various types of injuries and its forensic perspectives, as they were able to classify forensic injuries medically, legally and medico legally. Other 5% were lacking on this basic knowledge.

Knowledge and awareness on abrasions:

(Figure 1)- Abrasions are due to blunt force
Out of 115 students, 69% of students had knowledge on abrasions [refer figure 1]. Other 28% disagreed that abrasions are due to blunt force and 3% had no knowledge on abrasions. Most of them had awareness on abrasions common during road traffic accidents. Nearly half of the students were aware that abrasions do not affect both superficial and deep layers of skin. Whereas others lacked this information. 87.8% of the students had knowledge on pattern of abrasions and also agreed that time since abrasion can be identified by colour of scab. Only few disagreed that pattern of abrasion helps in identifying type of weapon and, that time since abrasions can be known with colour of scab. Other percentage of students didn’t have knowledge.

Knowledge and awareness on contusions:

(Figure 2)- Contusions causes bleeding

Knowledge and awareness on contusions were present only among of 49 students and 50% said contusions does not cause bleeding [refer figure 2]. Three by forth of students approximately had knowledge on ectopic contusions and minimal number of students lacked knowledge. Only 17.3% of students knew that bridging of tissue is not seen in bruise. Other 47.8% believed it and 26% didn’t know that it is only seen in laceration.

Knowledge and awareness on lacerations:

(Figure 3)- Lacerations involve all the layers of skin
Knowledge about lacerations was present among 75.6% of students [refer figure 3]. Others disagreed and few didn’t know that lacerations involve all layers of skin. Only 69.5% of students were able to differentiate between lacerations and incised wounds and believed that lacerations can lead to produce scar. Other few students were not able to differentiate between lacerations and incised wounds and 20% had no awareness and 20% disagreed the fact that lacerations will produce scar.

Knowledge and awareness on hesitational cuts:

(Figure 4)- Hesitational cuts are suicidal in nature

91% of students had good understanding on hesitational cuts [refer figure 4]. Only 5 out of 115 students disagreed that hesitational cuts indicates the suicidal nature of injury and believed that it’s homicidal. 6 students didn’t know what hesitational cuts are.

Knowledge and awareness on grievous hurt:
Only 70% of students gave correct responses on grievous hurt, [refer figure 5] where, few disagreed that emasculation comes under grievous hurt and some lacked knowledge on grievous hurt. And only 35.6% were known that abrasions do not come under grievous hurt while other students accepted it.

Knowledge and awareness on other types of mechanical injuries:

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<th>Incised Wounds</th>
<th>Homicidal Cut Injuries</th>
<th>Differentiating Chop Wounds From Cut Wounds</th>
<th>Types Of Stab Wounds</th>
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<td>CORRECT RESPONSE</td>
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<td>80%</td>
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<td>INCORRECT RESPONSE</td>
<td>18.2%</td>
<td>12.1%</td>
<td>71.3%</td>
<td>8.6%</td>
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<tr>
<td>DON’T KNOW</td>
<td>10.4%</td>
<td>7.8%</td>
<td>18.2%</td>
<td>2.6%</td>
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</table>

(Table 1)- Other types of mechanical injuries

71.3% students gave correct response as incised wounds are not due to sharp force whereas few gave incorrect responses and somewhere not aware.

Cut in genitalia are usually suicidal was disagreed by 80% of students and were correct about it.

Perpendicular strike due to heavy cutting weapon indicates chop wounds was disagreed by only by less percentage of students and nearly three forth students agreed it and gave incorrect responses and so was not able to differentiate between chop and cut wounds.
Penetrating and perforating wounds are caused by pointed weapons was known by many of students, minimal population disagreed and few lacked knowledge and awareness [refer table 1]

**Discussion**

The definition of physical injury in the forensic medicine is “damage to any part of the body due to the deliberate or accidental application of mechanical or other traumatic agent” [1]. The purpose of this assessment and documentation is establishing how injury is caused. As mistakes happen often, it is every doctor’s work to acquire knowledge on different injuries and their interpretation. Wounds are generally classified according to the method of caution and appearance. Each and every wound has different characteristics. These characteristics indicate the type of weapon. It is important for every medical practitioner as they often deal with medico-legal aspects of injuries throughout their practice.

A study done by, Barek A, Haque ST. [2] concluded that, the term hurt, injury and wound have almost the same meaning and some differences as well. They usually mean any damage to any part of the body or bodily harm caused by application of violence. Injury also includes any harm to the mind, reputation and property, and hurt includes bodily pain, disease or infirmity. Clinically a wound is produced when there is breach of anatomical continuity of the skin or mucous membrane with or without damage of the underlying tissues.

According to this study, knowledge and awareness on injuries were good among medical students. 115 medical students from Saveetha Medical College, Chennai, Tamil Nadu underwent this study. Out of which 95% of students had basic knowledge on injuries. There was a minimal lack of knowledge on contusions and grievous hurt and differentiating cut and chop wounds. This study mainly included mechanical injuries like abrasions, contusions, lacerations and other injuries like incised, cut, and chop wounds. Knowledge on medico-legal importance of each injury, hesitational cuts and grievous hurts was also present. In a similar study done by Rajeshree Rajendra Gaware et al., [3] 128 medical students participated, where knowledge and awareness on various injuries and its forensic perspectives were fairly good among medical students. Knowledge on its importance was present among 87.5% of the students. Knowledge on different types of injuries were present among 84.37% of students. But the knowledge on history taking were lacking among students. Thus understanding these injuries plays vital role among healthcare workers.

This study also shows that, students are having some knowledge and awareness about injuries but it should be improvised among them to make them fully aware about injuries. Similarly, Giannakopoulos GF et al., [4] done study to assess the knowledge of inflicted injuries of domestic violence and found that it should be improved among healthcare providers.

It is every medical professional’s duty to develop skills to assess and interpret different injuries. Another study done by Payne-James JJ, Hinchcliffe J. [5] says that, assessment and documentation about different injuries establishes the cause for the injury. They are rarely done correctly, so these skills should be within the remit of any medical professional. As there are many factors involved in interpretation of injury, it can be successfully done by forensic expertise. It can be undertaken by referring different documents like chart mapping, written descriptions or photographs.

The most common reason why medical evidence on injuries given in court is disagreeable is, confusing about the terms used by doctors and the inappropriate or inaccurate description of a wound, for example, documenting that the wound is laceration to describe cut wound caused by some sharp force such as a knife, when the wound is in fact, an incised wound [7], thus concludes that for proper differentiating and understanding various injuries, knowledge and awareness about it must be included among medical students which will surely help in future for better documenting of evidence. A comparative study done by, Reijnders UJ, Giannakopoulos GF, de Bruin KH.[6] concluded that, the injury assessment scores varied from good to blank in various participant groups such as forensic physician, emergency room physicians and nurses and medical students. So training in this field of medicine remains compulsory in all fields.

**Conflict of Interest:** Nil

**Source of Funding:** Self

**Conclusion**

The knowledge and awareness on various injuries is very important for every medical student and practitioner for recognizing and differentiating it. All types of
injuries have their own medico-legal importance which helps to clearly depict and for documenting them. Such reports certified by medical professionals only will help in judgment in the courts of law. The medical students were having good knowledge of the forensic perspectives of the injury, but certain areas shows lack of awareness. Thus it needs to be improved and the students should gain more understanding of injuries and their medico-legal importance because as a medical professional they are constantly exposed to plenty of injury cases.

**Ethical Clearance:** Obtained from institutional ethical clearance board

**References**


A Cross Sectional Study on the Awareness and Practice of Road Safety Measures among the Medical Students in Chennai

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Abstract

Background: The global epidemic of road traffic accidents is still increasing. The present study was aimed to assess the level of awareness regarding road safety among the study participants and to study the behaviour patterns while using motorised vehicles among the study participants.

Methods: The present cross-sectional study was conducted in Saveetha medical college and hospital, Chennai. A total of 125 students were studied. The study period was from 21st January 2019 to 30th March 2019.

Results: In total, 125 medical students were studied, ranging from 1st to final year M.B.B.S. of age 19-24 years; the mean age being 21.4. Only 65(52%) participants wear helmet and only 22(17.6%) participants interpreted Gap-In-Median correctly.

Conclusions: The awareness regarding road safety measures among the study group was satisfactory but interpretation of the road signs was not satisfactory -only 17.6% interpreted them correctly. The behavioural patterns among medical students are not satisfactory-only 52% of students wear helmet while riding, only 49.6% wear seat belts always while 41.6% wear seatbelts sometimes and 24% don’t follow lane rules while driving.

Keywords: Road safety measures, Undergraduate students, Awareness, Behavioural patterns.

Introduction

A road traffic accident (RTA) is any injury due to crashes originating from, terminating with or involving a vehicle partially or fully on a public road. It is projected that road traffic injuries will move up to the third position by the year 2020 among leading causes of the global disease burden [1]. In India, motor vehicle population is growing at a faster rate than the economic and population growth [2].

It has been estimated that unless immediate action is taken, road deaths in India will rise to the fifth leading cause of deaths by 2020 [3]. It may result in an estimated 2.4 million fatalities per year. Developing countries like India face the double burden of already existing communicable diseases and increasing burden of non-communicable diseases including RTAs [4]. Moreover, since it is affecting the younger generation of India, the future assets of the country are turning into a liability which is not ideal for the progression of our developing country to a developed one.

Road traffic accidents cost most countries more than 3% of their gross domestic product. Injuries sustained during RTAs cause considerable economic losses to individuals, their families, and to nations as a whole. The losses incurred are from the cost of treatment as well as lost productivity of those killed or disabled by their injuries, and for family members who need to take time off work or school to take care of the affected family member. An increase in average speed is directly related both to the likelihood of a crash and to the severity of the aftermath. Road traffic injuries can be prevented.
The 2030 Agenda for the Sustainable Development has set a lucrative target of halving the global number of fatalities and injury from road traffic accidents. This requires a hand from multiple modalities such as police, transport, education, medical, and actions that address the safety of vehicles, roads, and road users. Major interventions such as safer infrastructure and stressing the importance of road safety features in the transport planning, improving the safety of vehicles by enhancing the protective features, improving post-accident medical care for victims of crashes, making and enforcing laws relating to important risk aspects, and more importantly improving the awareness among the public. Simple measures such as following the road safety measures properly and also having the basic awareness about road safety can effectively reduce the impact of RTAs. On top of this another survey stated that in the last decade alone, India lost 1.3 million people to preventable road crashes and another 5.3 million have been left disabled for life [5].

The present study is aimed to assess the level of awareness regarding road safety among the study participants and to study the behaviour patterns while using motorised vehicles among the study participants. It is a questionnaire based retrospective study.

Methods

The present cross-sectional retrospective study was conducted in Saveetha medical college and hospital, Chennai with the aim to study the awareness of students on road safety and the road traffic accidents associated with them. Undergraduate MBBS students were included in the study, both male and females took part in this study. Students without driving licenses were included in the study as violation of the road traffic rules were incorporated in the questionnaire. Students belonging to the age group of 19 to 24 years were targeted in this study and a total of 125 students were studied. Mean age of the study group was 21.4. The study period was from 21st January 2019 to 30th March 2019.

A pretested semi-structured Questionnaire including general information and specific questions regarding awareness and behavioural patterns about road safety measures was given to the study participants and were instructed to fill them, the questionnaire validation had been accounted for by esteemed professionals and filled Questionnaire were obtained from the participants. A total of 27 multiple choice questions were included and the participants were asked to answer every question. Every possible measure was taken so as to uphold the confidentiality that was promised to the participants in the first place. No personal information regarding the participants was let out and the participants were also provided with the contact number and address of the principal investigator in the information sheet which the participants were given the liberty to take it with them if they wish.

Any queries regarding the study was well appreciated and addressed to in the best manner possible. The participants were also given the liberty to pull out of the study whenever they wanted if they felt unsafe or uncomfortable with the study. After obtaining the data, it was entered in excel spreadsheet and was analysed using SPSS software and frequencies and percentages were obtained.

Results

In total, 125 medical students were studied, ranging from 1st year to 4th year MBBS of age 17-25 years. 72(57.6%) participants were 19 years old, 30(24%) participants were 20, 7(5.6%) participants were 21, 2(1.6%) participants were 23 and 14(11.2%) participants were 24 years old. 62(49.6%) participants were male and 63(50.4%) participants were female (Refer Fig 1).

(Fig 1) Gender of Participants

Out of 125 participants, 64(80%) participants did not attend any programs regarding road safety measures and 36(45%) participants had attended. The response to the question whether the participants always wear their helmets was equally split among yes and no. To the question “From which side are you supposed to overtake vehicles” almost 63% participants gave the correct answer which is from the right side.
From the data collected, 31 (24.8%) participants said they use mobile phone while driving, 94 (75.2%) participants said they don’t. Majority of the participants told they do not always drive below the speed limit mentioned on the signboard. Another alarming result was the number of people who drive vehicles without obtaining their driving license, 74 (59.2%) participants answered that they had driving license and 51 (40.8%) participants answered no. When asked the question regarding the importance and necessity of wearing seatbelt’s or helmet more than 90% participants answered yes which is satisfactory. 110 (88%) participants answered no to the question “Have you ever driven a vehicle under the influence of alcohol?” and 15 (12%) participants answered yes.

Among the participants, 58 (46.4%) participants answered 0.03% to the question “What is the permissible blood alcohol content for drivers in India” which is the correct value and the rest 67 (53.6%) participants answered wrongly. The results obtained regarding getting apprehended by the police for flouting road rules were meagre. 97 (77.6%) participants answered that they always follow the lane rules while driving whereas the rest 28 (22.4%) participants answered in the contrary. 95 (76%) participants kept track of their vehicles condition and service records whereas the rest 30 (24%) participants did not. A good chunk of the study group had violated the traffic signals at least once in their lifetime. (Refer table 1).

<table>
<thead>
<tr>
<th>Questions</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Have you ever been caught by the police for drunk and drive</td>
<td>6 (4.8%)</td>
<td>119 (95.2%)</td>
</tr>
<tr>
<td>Do you always follow lane rules</td>
<td>97 (77.6%)</td>
<td>28 (22.4%)</td>
</tr>
<tr>
<td>Have you ever skipped a signal</td>
<td>51 (40.8%)</td>
<td>74 (59.2%)</td>
</tr>
<tr>
<td>Do you keep track of your vehicles condition and service records</td>
<td>95 (76%)</td>
<td>30 (24%)</td>
</tr>
</tbody>
</table>

Identification of road signs was poor only a handful answered correctly. 95(76%) participants felt that drinking and driving was more dangerous than texting and driving. When asked who was the most influential person for a teen driver, mixed results were obtained (Refer fig 2).

(Fig 2) Impact of Social Circle on Driving

92 (73.6%) participants answered that they always stop their vehicles within the stop line at the signal whereas the
rest 33(26.4%) participants answered they don’t always stop before the line. When asked to give a response on the maximum number of people who could travel safely on a two wheeler 106(84.8%) participants answered 2, 12(9.6%) participants felt it was 3, 6(4.8%) participants felt it was 4 and only 1(0.8%) participant felt it was 1. 92(73.6%) participants were aware of the stipulated hand signals to indicate slowing down, stopping, turning or overtaking and 33(26.4%) participants weren’t aware of the hand signals. 50(40%) participants felt the police had the right of way over other vehicles on the road, 42(33.6%) participants felt it was the pedestrians, 22(17.6%) participants felt it was the driver themselves and the rest 11(8.8%) participants felt it was animals. On giving the description about a road sign and asking to identify what modality it belonged to this was the response (Refer table 2).

<table>
<thead>
<tr>
<th>Knowing your Road Signs</th>
<th>Guidance</th>
<th>Regulation</th>
<th>Warning</th>
</tr>
</thead>
<tbody>
<tr>
<td>White characters on blue in general/ On Green in express ways</td>
<td>82 (65.6%)</td>
<td>35 (28%)</td>
<td>8 (6.4%)</td>
</tr>
<tr>
<td>Black characters and symbols on yellow diamond</td>
<td>27 (21.6%)</td>
<td>59 (47.2%)</td>
<td>39 (31.2%)</td>
</tr>
<tr>
<td>Red or Blue circle, depending on prohibition or regulation</td>
<td>16 (12.8%)</td>
<td>30 (24%)</td>
<td>79 (63.2%)</td>
</tr>
</tbody>
</table>

**Discussion**

Road traffic accidents (RTA’s) are the result of many factors related to bike, car, driver and the nature of the road. Though the bike, car and road contribute to some extent, driver errors remain the most significant factor in increasing or decreasing the rate of RTAs [6].

While India contributes only a meagre 1% of the world’s vehicles, it accounts for more than 10% of global RTAs associated fatalities – the most in the world. According to the 2015 report on ‘Road accidents in India’, by the Transport Research Wing (TRW) of Ministry of Road Transport and Highways (MoRTH), 1,46,133 fatalities occurred in RTAs in 2015 which included a count of 12,589 children. This count is not only the highest that India has ever recorded in history, but it represents a 53.9% increase over the last decade, and nearly a ten-fold increase since 1970 [5].

The present study was conducted to assess the awareness and behaviour patterns regarding road safety measures among under graduate dental students. The mean age of the study participants was 21.4 and there was almost equal representation for both males and females. A study previously conducted reported that 67.3% were males and 32.7% were females [7,8]. Awareness of road safety measures among the participants was good. This may be due to the increase in campaigning through mass media. But only 79(63.2%) know that overtaking must be from right side only. This may be due less addressing of this issue in comparison to the other measures.

In the interpretation of traffic signs, the knowledge of the participants was not satisfactory. Only 22(17.6%) interpreted all the road signs correctly. Similar finding was observed by Gharaibeh et al in a study conducted in Saudi Arabia [9]. Regarding the behavioural patterns, nearly 40.8% of the participants were not having a driving license. 48% of the study participants don’t wear helmet always and 12% were involved in drunken driving.

Our findings were similar to the observation in the study conducted by Swamy et al in Chandigarh [10]. Use of seat-belts was one of the most cost effective way to prevent RTA related morbidity and mortality [11]. Thirty one (24.8%) participants admitted that they used mobile phones without hands free devices while driving. Furthermore, nearly seventy one (56.8%) participants agreed to having exceeded speed limits while driving. Almost similar findings were reported by Kulkarni et al from South India [12]. Janlert et al also reported 14% of
the riders who met with an accident had drunken driving [13]. 28 (22.4%) participants answered they do not follow lane rules while driving, which was similar to the results obtained in a previous study [14].

An alarming 41% participants answered that they blow the red light when they are in a hurry which is a significant rise in percentage when compared to a previous study which stated that only 28% people believe in breaking the signals if they are in a hurry.

Almost 41% participants said they maintained their vehicles in prime condition and were happy with that while a previous study stated that 57% participants were willing to pay more to improve the condition of their vehicles in order to make it more foolproof on the roads [5]. The behaviours concerning mobile usage and over-speeding while driving are highly dangerous to the driver and to the public. Hence, these behaviour patterns need to be addressed through proper legislative and educative measures.

From the results of the current study it is evident that the road safety rules aren’t followed up to the mark yet. Still there is a high incidence of young people driving vehicles without passing their driving tests, people using mobile phones on the road and high speeding on roads just for the fun of it. The current study has also cemented the fact that usage of helmets and seat-belts are still below par. One more alarming fact is that majority of the study group didn’t know what the basic sign boards meant which seems to be the most worrying aspect.

Practices relating to road safety should be encouraged to reduce the morbidity and mortality related to road traffic accidents. For young drivers to have a change and practice safe driving is by educating them the importance of having goals to drive and not to drive for sheer fun [15].

Conclusion
From the study it is pretty evident that the awareness on road safety measures among the study group satisfactory but interpretation of road signs was not up to the mark -only 17.6% interpreted the road signs correctly which is very poor. The behaviour patterns among medical students are not satisfactory-only 65% of students wear helmet while riding, only 49.6% wear seat belts always while 41.6% participants answered sometimes and 22.4% participants don’t follow lane rules while driving. The efforts for increasing road safety measures through signboards, posters and mass media should be strengthened to reduce the morbidity and mortality in relation to road traffic accidents. Further research needs to be conducted regarding this topic to assess the current situation regarding road safety measures across the various modalities and aspects of populations. Awareness generation and orientation towards road safety issues among the students should be done through periodic trainings. Strict enforcement of laws and periodic organization of traffic awareness campaigns are essential for checking out risky practices in driving and thereby can result in decreasing the burden of road traffic accidents.

Conflict of Interest: Nil.

Source of Funding: Self Funding.

Ethical Clearance: Obtained from institutional ethical clearance board.

References


A Study on Awareness of Breast Cancer among Nursing Students

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Abstract

Background: Breast cancer is the most common type of cancer in women worldwide and its incidence is increasing in many countries. Breast cancer could happen to any woman irrespective of her education, social or cultural background. The lack of knowledge and incorrect perception about breast cancer prevention among females are responsible for the negative perception of the curability of cancer detected early and of the efficacy of the screening tests. It is, therefore, important to assess the level of awareness of risk factors in our communities. Therefore the present study is aimed to assess the awareness of breast cancer among nursing students.

Methods: The present cross-sectional study was conducted among nursing students of SAVEETHA MEDICAL COLLEGE AND HOSPITAL, CHENNAI. A total of 150 nursing students were included in the study with their prior consent. The study period was from 21st January 2019 to 30th March 2019.

Results: Our results showed insufficient knowledge of breast cancer among nursing students even though majority of them have heard of it. The results indicated the need for increased breast cancer awareness among university students.

Conclusions: Our study participants did not have adequate knowledge on risk factors and symptoms of breast cancer. The implications of this study are to emphasize the need to teach nursing students breast cancer awareness and detection of breast cancer in the early stage their undergraduate courses.

Keywords: Breast cancer awareness, Nursing students, knowledge, Consequences, Attitude.

Introduction

Cancer is becoming a leading cause of death worldwide [1]. Breast cancer is the most common type of cancer in women worldwide and its incidence is increasing in many countries [2]. It is also the second leading cause of cancer death in women after lung cancer. Breast cancer could happen to any woman irrespective of her education, social or cultural background [3]. Breast cancer occurs when there is abnormal breast cell growth. Most common breast cancers include invasive ductal carcinoma and invasive lobular carcinoma or in other cells or tissue within the breast. Breast cancer signs may include a lump in the breast, a change in breast shape, dimpling of the skin, fluid coming from the nipple, a newly inverted nipple, or a red or scaly patch of skin.

According to the World Health Organization report in the year 2011, it is estimated that worldwide over 508,000 women died in 2011 due to breast cancer [4]. Almost 70% of women with breast cancer are aged over 50 years, and only 5% are younger than 40 years old [5]. There is marked geographical variation in incidence rates, being highest in the developed world and lowest in the developing countries in Asia, Middle East, and Africa. The global incidence of breast cancer is rising, particularly in developing countries that formerly had a low incidence [6]. The incidence of breast cancer is
increasing due to increase life expectancy, increase urbanization and adoption of western lifestyles in the developing world.

Therefore, it is important that emphasis on prevention and early diagnosis of breast cancer are employed everywhere. One of the major difficulties regarding breast cancer relates to the lack of patients’ awareness about the disease. The negative perception of the curability of cancer detected early and of the efficacy of the screening tests are due to the lack of knowledge and incorrectly held beliefs about breast cancer prevention among females. It is, therefore, important to assess the level of awareness of risk factors in our communities. The survival rates of breast cancer have increased, and the number of deaths associated with this disease is steadily declining, largely due to factors such as earlier detection, a better approach to treatment and a better understanding of the disease. Breast cancer can be detected at an early stage through a combination of monthly breast self-examination (BSE), regular clinical breast examinations and annual mammography beginning at the age of 40 years, are the best ways to limit morbidity and mortality associated with breast cancer [6]. Early detection of breast abnormality is an essential factor. This is a challenge for health care professionals, especially nurses; contribute a crucial role in providing breast cancer awareness to women. Nursing students are the future nurses who will have the opportunity to encourage and influence women to be breast aware [3]. Therefore this study is aimed to assess the awareness of breast cancer among nursing students.

**Materials and Method**

This study was conducted among nursing students of SAVEETHA MEDICAL COLLEGE AND HOSPITAL, CHENNAI. A total of 150 nursing students were included in the study with their prior consent. The study period was from 21st January 2019 to 30th March 2019. Institutional Ethics Committee (IEC) clearance was applied for and obtained. All the participants who took part in the study were briefed on the study’s purpose, and requested to participate in it. The questionnaires were distributed after describing the purpose of the study. The participants were assured that participation was voluntary, and confidentiality would be maintained. The study was done by using a cross sectional and a semi-structured questionnaire which included questions related to

1. Awareness regarding breast cancer
2. Knowledge regarding breast cancer.
3. Attitude towards risk factors for breast cancer.

Informed consent was obtained from the study participants. After collecting data, the data was edited and tabulated on Microsoft excel sheet before data analysis. The data was then analyzed and the percentage were obtained and chi square test was applied.

**Results**

In total, 150 nursing students were studied, ranging from 1st year to final year nursing of age 17-22 years; the mean age being 18.73. Out of 150 participants, 127(84.7%) belong to < 20 years and 23(15.3%) belong to > 20 years. 32(21.3%) belong to 1st year, 102(68%) belong to 2nd year, 7(4.6%) belong to 3rd year and 9 (6%) belong to final year.

Out of 150 participants, 148 (98.6%) have heard of breast cancer and 2 (1.4%). Out of which 78 (52%) have heard of breast cancer through books, 43 (28.6%) through media, 22 (14.6%) through hospital, 5(3.3%) through conferences and remaining through friends and other sources. (Refer fig 1 & table 1)

**NURSING STUDENTS WHO HAVE HEARD OF BREAST CANCER**

(Fig 1)
VARIOUS SOURCES OF INFORMATION OF BREAST CANCER

<table>
<thead>
<tr>
<th>SOURCE OF INFORMATION OF BREAST CANCER</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Books</td>
<td>78</td>
</tr>
<tr>
<td>Media</td>
<td>43</td>
</tr>
<tr>
<td>Hospital</td>
<td>22</td>
</tr>
<tr>
<td>Conference</td>
<td>5</td>
</tr>
<tr>
<td>Friends</td>
<td>1</td>
</tr>
<tr>
<td>Others</td>
<td>1</td>
</tr>
</tbody>
</table>

(TABLE 1)

Out of 150 participants, 19(12.6%) have family members suffering from breast cancer and 131(87.4%) do not have family members suffering from breast cancer. (Refer fig 2)

FAMILY MEMBER OF NURSING STUDENT HAVING BREAST CANCER

Out of 150 participants, major cause of death in women diagnosed with breast cancer was thought to be due to lack of awareness by 99(66%), due to low socio economic status by 23(15.3%), due to poor access to health care by 20(13.3%), due to hesitation by 8 (5.3%).(Refer fig 3)
Out of 150 participants, 22(14.7%) have attended breast cancer awareness programme and 128(85.3%) have not attended breast cancer awareness programme. 98(65.4%) were aware and 52(34.6%) were not aware of consequences of breast cancer if left untreated. (Refer fig 4 & 5)

STUDENTS WHO HAVE ATTENDED BREAST CANCER AWARENESS PROGRAM
Discussion

Breast Cancer is the most common cancer in women in India [7]. Breast cancer is overlooked by many till a near and dear one suffers from it. So this results in most people presenting only when symptomatic, and on an average, most ‘symptomatic’ cancers are stage 2B and beyond (significant numbers in stages 3 and 4). So the breast cancer patients tend to survive for shorter time than their western counterparts. In the Western countries, most of the breast cancers (read more than 75%) present in stages 1 and 2, resulting in good survival; and there is an ever increasing numbers of patients presenting with mammography detected cancer, with no symptoms. India has to reach this achievement, and it is only with aggressive promotion of screening and awareness and proper treatment that India will achieve this; and will take at least a few decades to reproduce similar results [8]. The main focus of the study was to assess breast cancer awareness and knowledge among university students.

The evaluation of public awareness, attitudes and practice of BSE is of important for the successful implementation of breast cancer control activities. There are no known proven means to prevent breast cancer, which increases our reliance on the methods for early detection in order to improve patient outcomes. The main motive of breast cancer awareness program is to promote and develop awareness about the importance of its early detection. Our overall results, projected a general lack of breast cancer awareness and knowledge among university students irrespective of their gender, marital status, years in university and nature of high school attended even though 148(99%) have heard of it. In the current study, of the 148 participants who have heard of breast cancer, 52% obtained their information through books and only less than 1% obtained their information from friends which is in contrast to the study conducted among female Jordanian students in which 51.8% obtained their information from friends [6]. This may be due to hesitation among the students.

In contrast to a study done by Mafuvadze et al who reported 72% of university students reporting knowledge of someone with breast cancer in the USA, in our study, less than 15% of participants reported knowledge of someone with breast cancer [9]. Our study similar to study among female Jordanian students showed that more than 80% participants were aware that BSE might help in early detection of breast cancer [6]. Our study showed
that only 14.7% has attended breast cancer awareness program and more than 85% have not attended any such programs. This might also be the reason for increased incidence of breast cancer. Therefore, more breast cancer awareness programs should be developed in universities including lectures, seminars, workshops and on hands training.

**Conclusion**

Our study participants did not have adequate knowledge on risk factors and symptoms of BC. However, the knowledge about prevention and early detection was observed among majority with a healthy attitude among nurses towards BC.

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Our study participants did not have adequate knowledge on risk factors and symptoms of breast cancer. This study shows the need to teach nursing students about the breast cancer awareness and early detection of breast cancer in their undergraduate courses. In addition, the provision of regular programs are necessary to increase and build up the confidence and skills of nursing students in BSE. This is the primary step toward increasing awareness about breast cancer and encourages early adoption of breast screening initiatives. Such workshops are recommended for the nurses working in primary health care settings. The nurses would be the advocates to promote breast awareness and BSE among people and it is important that they should be aware.

**Conflict of Interest:** Nil

**Source of Funding:** Self

**Ethical Clearance:** Obtained

**References**

Assessment of the Feedback Questionnaire from Students for a Weekend Lecture

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Abstract

Competency Based Medical Education (CBME) and Curriculum Implementation Support Program (CISP) are gaining a lot of momentum in India. Lecture still remains as a very important large group teaching method. Attendance of the students was seen to be reduced in weekend lectures. The primary objective of this research work was to estimate the understanding of learning objectives of the previous weekend lecture; and to get the students’ feedback on ‘status of attendance’, ‘relevance of weekend lectures’, ‘acceptance as adult learners’ and ‘marking proxy attendance in lectures’. The secondary objective was to find the association of the answers with that of ‘status of attendance’. The tertiary objective was to check if any association existed between students’ responses of ‘relevance of weekend lectures’, ‘marking of proxy attendance in lectures’ and ‘acceptance as adult learners’. A feedback questionnaire tool post validation was conducted unannounced following the weekend lecture and students voluntarily participated in the same – sample size was 82.

The students who had attended the lecture had given the maximum number of correct responses. The largest group of responses were ‘not answered’ and they belong to those who had not attended the lecture. All these interpretations of associations were found to be highly significant statistically - p < 0.001. There was an association between responses of ‘acceptance as adult learners’ with that of ‘marking proxy attendance in lectures (as bad attitude)’ - p < 0.049.

Key words: Adult Learner, Attendance, CBME, Feedback, Interactive Lecture, Pedagogy

Introduction

Competency Based Medical Education (CBME) is right now booming in India, and the doctors are mandatorily being teacher trained for the same. The Board of Governors (BOGs) through the Academic Cell are now focussing on Curriculum Implementation Support Program (CISP) very seriously for all the colleges in India; i.e. practical real time implementation of the new CBME curriculum. Nodal Centres and Medical Education Departments are responsible for carrying forward this vision of the BOGs.¹ In light of these recent developments in our country, while taking a look into the new curriculum; it was understood that CBME is actually a blend of traditional teaching methods along with the newer training methodologies. Under the section of Teaching-Learning (TL) Methods, in the new curriculum ‘Lecture’ was very much a part of the large group teaching methods. It is impossible to conflict the statement that Lecture has been the most common form of knowledge delivery TL method.²³ Small group teaching which is being now over-emphasized will be replacing most of the lecture hours; but even then there are earmarked lecture hours for each subject in the new CBME curriculum.
MCI expects medical colleges to take advantage of newer TL methods because one single approach is unlikely to suit for all learners, in terms of learning styles and preferences. However when it comes to scrutiny of attendance for lectures, a work was done abroad by Mattick and associates regarding attendance of students for non-compulsory lectures. They found the reason for diminishing attendance of students; as and when they progress through academic years, is their increasing confidence in the subjects or because of their increasing confidence as a result of learning through other TL methods. The researchers also saw a higher attendance percentage in lectures scheduled at lunch time because the students are having either morning or afternoon classes, and hence are more likely to be there in the teaching campus.4

The education system cannot be reformed and polished unless the feedback from the stakeholders are utilised for the same. A learner’s feedback can be reliable, valid and used genuinely in enhancing the quality of student learning and experience. Feedbacks can be questionnaires (which can be judgemental) or reflections (open ended). Even student diaries can be taken as one of the confidential reflections of a teaching learning environment.5 More than for the system, feedback can influence (improve or worsen) the learning behaviour of students and the skill development. It is perhaps understood that action without feedback is completely unproductive for any learner and this is even more apparent when feedback happens without any subsequent changes in actions.6,7

The data collected from feedback of learners is often considered very divisive, political and sensitive in an education system; and there exists a ‘tension’ between stakeholders (administrators, teachers and students) and the very purposes of collecting the feedback. This is because the demands of improvement and accountability are in conflict. Hence a perfect system/method of collecting feedback would maintain peace among the stakeholders.8

In the current research work, the following objectives were considered: The primary objective of this research work was to estimate in students the understanding of learning objectives of the previous weekend lecture (as answers); and to get the students’ responses/feedback on ‘status of attendance’, ‘relevance of weekend lectures’, ‘acceptance as adult learners’ and ‘marking proxy attendance in lectures’. The secondary objective was to find the association of the answers with that of ‘status of attendance’. The tertiary objective was to check if any association existed between students’ responses of ‘relevance of weekend lectures’, ‘marking of proxy attendance in lectures’ and ‘acceptance as adult learners’.

Materials & Method

It was decided to take an unannounced feedback of an interactive lecture that was conducted on a Saturday. Usually the attendance on such weekend lectures were found to be dropping low and there were few incidents of marking proxies too for the same. Following the Saturday 3-4pm lecture the same topic was further continued on Monday 1-2pm. The Monday session began for the same batch of students with a structured closed end feedback questionnaire that was completed in 10 minutes. The questionnaire was validated by the faculty members of the same subject and was modified accordingly post validation. The feedback questionnaire contained 15 points. Anonymity was ensured and students were given the freedom to opt out of the questionnaire completely. In the questionnaire six aspects were pertaining to the topic that was covered in the previous Saturday session and three were focussing on their attitudes and opinions (responses) of ‘relevance of weekend lectures’, ‘marking of proxy attendance in lectures’ and ‘acceptance as adult learners’. The other pointers were for recording their attendance of the Saturday session-with reason for absence, feedback of the feedback questionnaire, sex, age and signature.

The class had an attendance of 82 students. This batch of students was chosen since they had attended the Saturday lecture. Hence it was purposive sampling as far as the batch was considered. The study was prospective in nature. All the students who had attended the Monday interactive lecture had taken part in the study; there were no exclusions/omissions. The sample size was all the 82 students. The study was a pilot work since there was no exactly similar research work in the existing literature. The statistical analysis was done using IBM SPSS 20.0. For all the continuous variables, the results are given in Mean ± SD and for categorical variables as percentage. To find out the association between two categorical variables, chi-square test was applied. A P-value < 0.05 was considered as statistically significant.

Results

In total, 64.6% of the students were having an
age above 20 years (mean was 19.82 ± 0.818) and 53 (64.6%) students were females. The name of the topic that was taken on Saturday was correctly answered by 33 (40.2%) students. The mnemonic (for muscarinic effects of A/C poisoning with organophosphates) was answered correctly by 30 (36.6%) students. The antidote/s was correctly listed by 25 (30.5%) students. The requisite of an end-point in antidote administration was answered rightly by 33 (40.2%) students, and as to how this end-point needs to be clinically confirmed was correctly written by 15 (18.3%) students. As the last pointer, 18 (22%) students wrote the correct expansion of High Performance Liquid Chromatography (HPLC).

It was seen that 46 (56.1%) students had attended the lecture session on Saturday. The reasons for ‘not attending’ as given by the remaining students were grouped into three categories – Went home, Unwell and Other appointments. Of these three, ‘Unwell’ category constituted the highest percentage. The response from students for ‘relevance of Saturday (weekend 3-4pm) lectures’ were grouped into three categories: Lectures are - Boring, Not Sure and Essential. The highest number of responses was for the group ‘Essential’ (38 students - 46.3%). The students were asked as to whether they were adult learners and 36.6% (30 students) of them said that they believe in same (andragogy). The next pointer was as to the reasons they feel they are adult learners or not; and the highest group of responses supporting andragogy was the fact that these students believe in concept of self-learning. At the same time, the maximum number of reasons supporting pedagogy was the fact that they ‘require teacher input’. The students were asked for their opinion in giving proxy attendance during such lecture hours and their responses were grouped into three categories – Bad attitude, Neutral and Good attitude. The highest response was in support for ‘proxy attendance being a bad attitude’ – 69.5% (57 students). A feedback of the feedback questionnaire was taken and 38 (46.3%) students had chosen the response – ‘interesting feedback questionnaire’. Miscellaneous comments were sought from the students as to in-general how they are finding the lectures and 27 (32.9%) students gave their comments. Out of these nine students - 11% (highest category of response) said that the interactive lectures are ‘interesting and good’.

Table one and two - the ‘answers from students’ were compared to their ‘status of attendance’; those who had attended the Saturday session had given the maximum number of right responses. The largest group of responses are ‘not answered’ and for all the six questions they are coming under the category of students who had not attended the lecture on Saturday. All these interpretations of associations were found to be highly significant statistically.

<table>
<thead>
<tr>
<th>Attendance</th>
<th>Name of Topic</th>
<th>Mnemonic</th>
<th>Antidotes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right</td>
<td>Wrong</td>
<td>N.A.</td>
</tr>
<tr>
<td>Yes</td>
<td>26 (56.5%)</td>
<td>7 (15.2%)</td>
<td>13 (28.3)</td>
</tr>
<tr>
<td>No</td>
<td>7 (20%)</td>
<td>3 (8.6%)</td>
<td>25 (71.4%)</td>
</tr>
<tr>
<td>P value</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.006</td>
</tr>
</tbody>
</table>
Table 2: Association of 2nd three sets of answers with that of attendance (N.A. – Not Answered)

<table>
<thead>
<tr>
<th>Attendance</th>
<th>End-Point</th>
<th>Clinical confirmation of End-Point</th>
<th>HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right</td>
<td>Wrong</td>
<td>N.A.</td>
</tr>
<tr>
<td>Yes</td>
<td>28 (60.9%)</td>
<td>3 (6.5%)</td>
<td>15 (32.6%)</td>
</tr>
<tr>
<td>No</td>
<td>5 (14.3%)</td>
<td>0</td>
<td>30 (85.7)</td>
</tr>
<tr>
<td>P value</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Responses of the ‘relevance of weekend lectures (boring/not sure/essential)’ were compared with that of ‘marking of proxy attendance in lectures (bad attitude/neutral/good attitude)’ and it was seen that responses of marking proxy as being a bad attitude was associated with that of weekend lectures being considered as essential; but this was not statistically significant. Similar an association was seen in the ‘not sure’ and ‘neutral’ categories respectively (statistically not significant).

The responses of ‘acceptance as adult learners’ was also compared with that of ‘marking proxy attendance in lectures’. There was a good association between being considering them self as an adult learner with that of considering marking proxy attendance in classes as a bad attitude (24 students – 80%); and it was significant statistically (p < 0.049). The non adult learners were similarly associated with the ‘neutral’ attitude to marking proxy attendance, with same statistical significance (Fig 1).

Fig 1: Association of ‘adult learners’ with ‘marking proxy attendance’ (AL – Adult Learner, NAL – Non-Adult learner)
Last but not the least; ‘acceptance as adult learners’ responses was compared with responses of ‘relevance of weekend lectures’. There was no obvious or strong association, and there was no statistical significance also for the same.

**Discussion & Conclusion**

The students have taken part with an open mind set and genuine interest to improve the system. So the teachers have their own share of responsibility by being transparent to identify the actions that need to be taken. In other words the educators are meant to take an active role rather than a passive receiver of feedback. When comparing the different methods in literature, this study was that of a rapid feedback method, and hence is less stressful and not too demanding from the students.5 It was also a closed end type of feedback session, thereby convenient for statistical summation, but on the other hand being a quantitative study and not a qualitative one (without focussed group discussions); the educator often will struggle to make sound reforms in the TL process.

The ‘name of the topic’ and the ‘importance of end-point’ were answered as higher percentages because the teacher had stressed on these two points in the previous class. But the ‘clinical confirmation of end-point’ was remembered the least among the take home messages, which could be because the students had not understood the relevance of the same; and this needs to be taken care in subsequent lectures. Out of the reasons that were sought for not attending the lecture, since ‘unwell’ was top scoring, the educators have to seriously take into consideration as to why the students are falling ill – whether it is to do with the poor food habits or unhealthy lifestyle prevailing at their residence/hostel.

It is to be taken with a positive stroke that the majority of them are considering themselves as ‘adult learners’, since this is the crux of the SPICES9 model of modern education system. Even the majority have opined that marking proxy attendance is a matter of shame and bad attitude. This category of students was also supporting the concept of self-learning. The importance of self-learning was already studied by the author in another research work involving only faculty members10, and now when the results of that work is read together with the students’ response in this work, everything is pointing in the direction of andragogy through self directed learning. The non-adult learners need to be encouraged by the educators to slowly start taking responsibilities on their own for their own benefits. The modus operandi of taking the attendance will be tightened up in the future lecture sessions.

Statistically all the answers for the six subject/lecture pointers were compared to the presence and absence status of the Saturday lecture. The largest category of the ‘unattended/not answered’ was coinciding with their absence in the previous lecture. Similarly the right answers were highest in students who had taken part in the Saturday Lecture. Being statistically significant this shows beyond any reasonable doubts that the students who had attended got definitely benefitted from the interactive lecture session on this particular topic. This also strengthens the fact that we cannot do away with lectures permanently. The responses for ‘relevance of weekend lectures’ with that of ‘marking of proxy attendance’ in class though had an association, cannot be affirmed for its veracity because of non-significant statistical interpretation. However the association of the responses of ‘acceptance as adult learners’ with that of ‘marking of proxy attendance’ was statistically significant and this shows the importance of adult learners being responsible for shouldering their learning responsibilities and also having the right attitude in a lecture session; including to not to mark proxy attendance.

**Acknowledgment:** We would like to thank the Professor and HOD of Forensic Medicine and Toxicology Department in AIMS, Dr. VV Pillay for his kind support in conducting this feedback questionnaire session in one of the interactive lectures of the subject. We would also like to thank the other faculty members in the same department for validation of the questionnaire tool.

**Conflict of Interest:** There are no conflicts of interest in this research work

**Source of Funding:** Nil

**Ethical Clearance** Ethical Clearance was not required, (as per the guidelines in ICMR) for any research work into medical education or curricula/teaching activities modification.

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Assessment and Examination of Female Rape Victims and Their Genital Injuries

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Abstract

**Background:** The prevalence of genital injury resulting from sexual assault has been an area of interest to the scientific and health care community. The present study was conducted to assess female rape victims and their genital injuries.

**Materials & methods:** The present retrospective study was conducted in the department of Gynaecology. It consisted of 28 female patients of rape victims. All suspected victims were examined in gynecology and forensic medicine department. A through vaginal examination was performed by gynecologist.

**Results:** Age group 20-25 years had 16 females and age group 25-30 years had 7 females and 30-35 years had 5 females. The difference was significant (P< 0.05). Tanner staging- hair stage II was seen in 8, hair stage III in 20, V-shaped notches were single in 4, multiple in 24. Notches were at 6 o clock position in 5, 9 o clock position in 6 and at both positions in 17. The difference was significant (P< 0.05).

**Conclusion:** The number of rapes is increasing day by day. A through gynecological examination is required to confirm the crime.

**Key words:** Rape, Gynecology, genital injuries, sexual assault.

Introduction

An estimated 876,064 rapes are perpetrated against women each year in the United States. Among injured female victims of sexual assault, one half of those in which the violence was reported received some type of treatment from a health care provider. The prevalence of genital injury resulting from sexual assault has been an area of interest to the scientific and health care community since the 1970s.¹ Investigators have, over the years, typically reported the prevalence and location of injuries that occur most commonly; however, less is known about other more subtle aspects of injury, such as the type, severity, and extent of the wounds. Sexual violence occurs throughout the world.²

Although in most countries there has been little research conducted on the problem, available data suggest that in some countries nearly one in four women may experience sexual violence by an intimate partner, and up to one-third of adolescent girls report their first sexual experience as being forced.³ Sexual violence has a profound impact on physical and mental health. As well as causing physical injury, it is associated with an increased risk of a range of sexual and reproductive health problems, with both immediate and long-term consequences. Its impact on mental health can be as serious as its physical impact, and may be equally long lasting. Deaths following sexual violence may be as a result of suicide, HIV infection or murder – the latter occurring either during a sexual assault or subsequently, as a murder of “honour”. Sexual violence can also profoundly affect the social wellbeing of victims; individuals may be stigmatized and ostracized by their families and others as a consequence.⁴ The present study was conducted to assess female rape victims and their genital injuries.
Materials & Method

The present retrospective study was conducted in the department of Gynaecology. It consisted of 28 female patients of rape victims. All subjects were informed about the study and written consent was taken. Ethical clearance was obtained prior to the study.

Data such as name, age, sex etc was recorded in performa. All suspected victims were examined in gynecology and forensic medicine department. A through vaginal examination was performed by gynecologist. Results thus obtained were studied. P value < 0.05 was considered significant.

Results

Table I Age wise distribution

<table>
<thead>
<tr>
<th>Age group (Years)</th>
<th>Number</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-25</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>25-30</td>
<td>7</td>
<td>0.01</td>
</tr>
<tr>
<td>30-35</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

Table I shows that age group 20-25 years had 16 females and age group 25-30 years had 7 females and 30-35 years had 5 females. The difference was significant (P< 0.05).

Table II Assessment of parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Number</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tanner staging- hair stage II</td>
<td>8</td>
<td>0.01</td>
</tr>
<tr>
<td>Hair stage III</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>V- shaped notches- single</td>
<td>4</td>
<td>0.02</td>
</tr>
<tr>
<td>Multiple</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Notches- 6 o clock position</td>
<td>4</td>
<td>0.05</td>
</tr>
<tr>
<td>9 o clock position</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Both</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

Table II shows that Tanner staging- hair stage II was seen in 8, hair stage III in 20, V- shaped notches were single in 4, multiple in 24. Notches were at 6 o clock position in 5, 9 o clock position in 6 and at both positions in 17. The difference was significant (P< 0.05).

Discussion

Sexual violence is defined as: any sexual act, attempt to obtain a sexual act, unwanted sexual comments or advances, or acts to traffic, or otherwise directed, against a person’s sexuality using coercion, by any person regardless of their relationship to the victim, in any setting, including but not limited to home and work. Coercion can cover a whole spectrum of degrees of force. Apart from physical force, it may involve psychological intimidation, blackmail or other threats – for instance, the threat of physical harm, of being dismissed from a job or of not obtaining a job that is sought. It may also occur when the person aggressed is unable to give consent – for instance, while drunk, drugged, asleep or mentally incapable of understanding the situation. Sexual violence includes rape, defined as physically forced or otherwise coerced penetration– even if slight – of the vulva or anus, using a penis, other body parts or an object. The attempt to do so is known as attempted rape. Rape of a person by two or more perpetrators is known as gang rape. Sexual violence can include other forms of assault involving a sexual organ, including coerced contact between the mouth and penis, vulva or anus. The present study was conducted to assess female rape victims and their genital injuries.

In this study, age group 20-25 years had 16 females and age group 25-30 years had 7 females and 30-35
years had 5 females. Typically, acute injuries of the external genitalia observed in child rape victims include lacerations, bruise, abrasion, redness, and edema of the posterior fourchette, labia majora, labia minora, hymen, or vulva. The hymen of a child is easily torn by the insertion of an adult penis, as the pre-pubertal physical structure of the hymen is relatively smaller than that of adults.\(^8\)

In a study conducted by Plichta et al\(^9\), the genital scars of pre-adolescent sexual abused victims were monitored for 10 years. Of 24 child victims who were raped, transection was observed in 12 victims. The transection of the hymen did not naturally heal unless they were surgically reconstructed.

We found that Tanner staging- hair stage II was seen in 8, hair stage III in 20, V- shaped notches were single in 4, multiple in 24. Notches were at 6 o clock position in 5, 9 o clock position in 6 and at both positions in 17. Campbell et al\(^10\) conducted a study on scar patterns in the genital organs of sexually assaulted female victims. Hymenal laceration occurred more frequently in adolescents than in adults, and the difference was statistically significant. Hymenal injury is more frequent in adolescents than in adults because of the lack of sexual and childbirth experience. Therefore, when a child is raped, hymenal injury is more likely to occur, or the severity of the injury is more serious in adolescents than in adults. Severe hymenal scars, such as deep notches of over 50% or transections, may remain permanently even after several years. Most child rape cases have a delayed disclosure of sexual abuse. Therefore, by the time medical professionals examine child victims, the injuries are most likely healed to a certain degree.

**Conclusion**

The number of rapes is increasing day by day. A thorough gynecological examination is required to confirm the crime.

**Conflicts of Interest:** The authors declare that there is no conflict of interest regarding the publication of this paper.

**Source of Funding:** Self

**Ethical Clearance:** Ethical clearance has been taken from Institutional Ethical Committee

**References**

Visum Et Repertum in the Evidencing Process of Rape in Indonesia

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Abstract

Rape is part of sexual crime in which its evidencing process is rather complicated if the victims do not directly report the case, or if the law-enforcing apparatus are not quick in handling it. *Visum et repertum* is a medical-aspect report from the doctor after a written demand from the law-enforcing apparatus of authority for the sake of the case investigation and examination in court. Thus, from this definition, *visum et repertum* can only be proposed by the law enforcers or the authorities such as the police department, the lawyers, or the judges in court to the special doctors who are in charge of that field. The lawyers and the judges may ask for the *visum* through the police department. This research uses a normative doctrinal method. The research results show that the evidencing process of rape as a criminal act of sexual crime has some different difficulties compared to other general criminal acts. This is because there needs to be *visum et repertum* which will uncover the perpetrator and the time of occurrence, which will ease the process of searching for and finding the perpetrator. The obstacle of *visum et repertum* is that its evidencing process is the same as a letter of evidence in the aspect of power.

**Keywords:** visum et repertum; evidencing; rape; Indonesia

Introduction

In obtaining adequate evidences of a criminal case, the law enforcers are often faced with complicated cases, which require special skills. They might not even have the skills as they are not in their expertise. Thus, there needs to be other elements in the law enforcement to make a criminal case clear. This is the importance of the experts’ role to find a complete material truth to complete the evidencing process of a case file, so that it may be judged and decided upon by the judge, as said by Soesilo.\(^{(1)}\)

Rape is a criminal action which is very different from other crimes. This is because the evidencing process require a special aspect, which is only understood by medical forensic experts. Rape is an action of violence and a crime of decency which attacks someone’s dignity. It is done by coercing someone to undergo sexual intercourse using violence or threat of violence. Because this intercourse must be proven on when it happened, and who did it, there needs to be information from experts of medical forensics to be expert witnesses in that case. They also need to make a report called *visum et repertum*. The expert witnesses will also be present in court if the case is under trial.

The aim of the expert information’s presence is to assist the law enforcers in the levels of investigation, lawsuit, and trial to give valid evidences and medical information which can be trusted regarding the victim’s condition, especially regarding evidences of sexual violence which comes with intercourse, both physical violence on the victim’s body and the threats of violence which preceed it.

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Visum et repertum is used to uncover rape cases as criminal cases with the aim to achieve truth. This will bring justice to the victims, as the visum et repertum is crucial in the medical forensics for evidence. This is the importance of visum et repertum for obtaining evidences.

The function of criminal law procedures according to JM. Van Bemmelen in Soedjono(2) are as follows:

1. To find truth of the accusation in the criminal code violation through the state instruments;
2. The effort to find the perpetrators who violated those regulations/to find the perpetrators of the action;
3. The effort so that the perpetrator of that action is caught, and if needed, they should be imprisoned;
4. The collection of evidences or bewijsmateriaal which have been obtained to uncover the truth, to be given to the judges, and to bring the defendant to the face of the judges;

The view of the criminal law procedures is carried out to obtain a material truth, which is according to the aim of the criminal law, which is to find the material truth of a case. This is different from the civil law, which only looks for a formal truth. In the other hand, the evidences are taken from the body of the victim, such as traces of saliva, sperm in the victim’s vagina, and evidences of violence such as wounds, bruises, etc. Such things are usually invisible to the eye. Only medical forensics can translate those evidences as well as to find them as carefully as possible, as opined by Idris and Tjiptomartono.(3)

There is a tendency in the Indonesian culture that the rape victims are considered as unclean and polluted creatures. This makes the victim, the family, the friends, and the acquaintances hesitate from reporting as they are ashamed. They are scared that the society will view them negatively. Thus, the report which is supposedly done as soon as possible is postponed. They may not even report until months or years. Thus, the evidences of rape are gone. This will make the evidencing process of that case difficult.

**Finding and Discussion**

The science of medical forensics calls visum et repertum only as visum. Visum is the latin word for visa, which means to see. Terminologically, visum means a sign from an evidence from anything found, agreed upon and validated. Meanwhile, repertum means to report. Thus, terminologically, it means whatever is found from the doctor’s examination to the victim or what has been validated by that doctor, as opined by Ranoemihardja.(4)

The report of that doctor, which is called visum, must be in the form of a written report from a forensics doctor who have done a medical swear, so that it may become a valid evidence in the court trials while judging the perpetrator, as explained by Ranoemihardja.(4) Visum et repertum, which is part of medicolegal has three kinds, in which according to Soeparmono(5) are explained as follows:

1. **Visum et repertum** for living people

   There are three kinds of this visum, which are:
   a. **Visum et repertum** for wounds/direct visum et repertum/ definitive visum et repertum

   The qualification of the wound written by the doctor on the conclusion of the visum et repertum is wounds of the first degree and group C wounds. The doctor is not allowed to write light violence wound as this is a legal term.
   b. **Temporary visum et repertum**.

   This visum needs further care and treatment, thus it may become an obstacle for the victim’s work.
   c. **Continued visum et repertum**.

   This visum is done when the victim’s wound is considered as healed. Another reason is when the victim is moved to another hospital, of if he/she is treated by a different doctor, or he/she goes home before the assigned time.

2. **Visum et repertum** for dead bodies.

   This visum is done if the victim is dead. Thus, the doctors undergo visum et repertum for dead bodies. The doctor writes the wound qualification in the conclusion of the visum et repertum.

3. **Expertise**

   This visum et repertum is specially done to check the condition of an item or the victim’s body part. For instance, this may be blood, sperm, saliva, body tissues, hair, bone, etc. There are parties who say that expertise is not part of visum et repertum.
In asking for a *visum et repertum* report to the doctors, especially if the victim is still alive, the authorities or the apparatus must take note of these things regarding the rape victim, as described by Soeparmono:(5)

1. The demand for *visum* must be done in a written form, as opposed to the oral form;
2. The demand for the *visum* must be done directly to the doctor, without any mediators, including the media of postal service, and/or through the victim or the family;
3. It is not a past phenomenon, as it is included in the confidential information of the doctor’s position;
4. The reason of why there must be the *visum*, and why he/she must be taken to the doctor;
5. The victim’s identity and the identity of the apparatus who asked for the *visum* and date of visum.
6. The victim must be directly accompanied by the police or the lawyer.

The main aim of investigation is to collect as much data as possible, regarding the matter, evidences, and correct facts regarding the phenomenon. Based on these facts, the investigators will try to reexplain and describe what had happened. The incomplete facts will be looked for to complete the imagery of the occurrence, so that it will be complete.

The process of a criminal case investigation by the investigators is then informed to the general plaintiff with the giving of the Notification Letter regarding the Start of an Investigation (SPDP) based on Article 109 paragraph (1) of the Criminal Code. After the evidences are collected, and the alleged perpetrator is found, then the investigator will assess the defendant carefully. The investigator will see if the evidences are enough, to be bestowed to the general plaintiff or maybe it is not a criminal act. If the investigators think that the occurrence is not a criminal action, thus the investigation is stopped for the sake of law.

Then, in Article 8 paragraph (3), if the investigation is finished, then the investigator will give the case files to the general plaintiff. The handover is carried out through two stages. *First*, in the first stage, the investigator will only handover the case files. *Second*, in the second stage, if the investigation is considered to be complete, the investigator will bestow the responsibility for the defendant and the evidences to the general plaintiff.

Then, in Article 110 paragraph (4) of the Criminal Code, the investigation is regarded as finished if within a 14 (fourteen)-day period, the general plaintiff does not return the investigation result files. Or, if before the determined period has ended, there has been a notification regarding that case from the general plaintiff to the investigators. After the investigation is regarded as finished, thus the investigator will bestow the responsibility regarding the defendant and the evidences to the general plaintiff, as explained by Prodjodikoro.(6)

The aim of an investigation is to obtain a decision from the general plaintiff, whether there is enough evidence to sue the alleged perpetrator. The criminal case process is a part of an integrated action of the law enforcement implementation. Between the investigation and the prosecution there is a strong relation. Even, the success of the prosecution in court trials cannot be separated from the results of the investigation.

The crime of rape means trying to coerce through violence, forcing with violence or overpowering. The definition of the criminal action of rape is wider than merely sexual intercourse. Yet, it may also happen in different forms, including the violation of another person’s human rights, according to Van de Tas.(7)

According to Soetanjo, what is meant by rape is an effort to force a man’s sexual desire to a woman, which violates the morals and law which apply. According to this definition, what is meant by rape on one side may be seen as an action of coercion to release one’s sexual desire. And, on the other side, it can be seen as an action of violation towards the norms and the social order, said Soebroto.(8)

The definition of rape above shows that rape is a form of an action in which a man forces his sexual desires towards a woman. Bothe morally and legally, this action violates the norms of morality and decency in the society. Thus, it is clear and it is even an obligation to categorize rape as a type of criminal action.

The provisions which regulate the forms of action and imprisonment of rape is called *verkrachting*, which is regulated in Article 285 of the Criminal Code. It is written in that article that, “Whoever, with violence or threat of violence, forces a woman to undergo sexual intercourse with him outside of marital relations, is deemed to have underwent rape, with the sanction
of imprisonment for a maximum of twelve years,” as written by Moeljatno.\(^{(9)}\)

In the material regarding the criminal act of rape in Article 285 of the Criminal Code, its elements, according to Chazawi\(^{(10)}\), may be described as follows:

1. The action: coercion
2. The manner, may be done through as follows:

With violence, with the threat of violence, with a woman who is not his wife, there is the action of intercourse

The action of coercion (dwingen) is an action which is done to another person by pressing the willingness of that person. This is so that the other person accepts the willingness of the enforcer. The point of coercion’s definition is doing something outside of another person’s willingness, which is against that person’s desire.

Satochid Kartanegara states that this action of coercion should be interpreted as an action which creates fear withing another person. The action which makes a woman “coerced” to undergo a sexual relation, must be categorized into the definition of “coercing” a woman to undergo sexual relations, even if that person undresses her own clothes, according to Marpaung.\(^{(11)}\)

According to Soesilo, the definition of violence is “using big power and physical strength illegally”. Meanwhile, according to Kartanegara, violence is “every action which uses physical strength which is not light yet rather hard,” according to Marpaung.\(^{(11)}\)

The understanding through the definition of violence in Article 285 is as follows. Violence is a manner or an effort to undergo (something abstract) which is targeted to another person to create an action using physical strength, which makes another person physically powerless. As said by Chazawi\(^{(10)}\) it may be done using wood, hitting, stabbing, etc.

Tirtamidjaja opines that sexual relations or sexual intercourse is the internal contact of the man’s and the woman’s genitals which usually causes pregnancy, there is no need for the excretion of sperm in the woman’s vagina, as said by Mapaung.\(^{(11)}\) Then, Anwar\(^{(12)}\) defines intercourse as the action of a penis’ penetration to the vagina. This penetration may be complete or incomplete, with or without ejaculation. Then, even though the action of intercourse happened in the past, it can still be seen by the science of medical forensics, through careful \textit{visum et repertum} according to Harahap.\(^{(13)}\)

The evidencing of the \textit{visum et repertum} results have the same power as the evidence of letters. The judge has the authority to determine the strength of each. If the judge believes that the \textit{visum} is truthful, thus the judge may give the verdict that the defendant is guilty. Thus, in the criminal case of rape, the accuracy of the doctor’s analysis in producing the evidence of \textit{visum et repertum} is crucial. This is the main point of the evidencing process, to ease the judge’s belief so that he/she believes in that evidence.

\section*{Conclusion}

Sexual violence in the form of rape is a criminal act. It does not only happen in developing countries, but it is also a disturbing problem in developed countries. It can be said that the evidencing process of this crime is rather different and more complicated compared to other crimes. Thus, there needs to be the involvement of different parties to handle this sexual violence case of rape. Usually, the law-enforcing apparatus involves the medicolegal element or medical forensics which has the special skills in uncovering the evidences of the rape case. Such evidences may then be used in the court trials. The police usually have trouble finding strong evidences. Another obstacle is the fact that the rape victims are usually reluctant to report their case. This is because in the local culture, such cases cause shame to the victim. That’s why, there needs to be special skills from the medicolegal, through \textit{visum et repertum} to obtain such evidences. The evidencing strength of the \textit{visum et repertum} depends on the judge’s belief. Thus, a weak \textit{visum} may be a strong evidence if the judge believes that it is truthful.

\section*{Ethical Clearence: Yes}

\section*{Conflict of Interest: No}

\section*{Source of Funding: Authors}

\section*{References}


Effectiveness of Conventional Physiotherapy Exercises Versus Kinesiotaping in Recreational Football Players with Plantar Fasciitis

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Abstract

Background: Plantar fasciitis is a disorder in which there is non-inflammatory structural breakdown of the plantar fascia. It is more common in sports that involve running and long-distance walking. Many of the literature suggest that there is 21.7% of prevalence rate of plantar fasciitis in individuals who play sports, especially in football players who uses studs/cleats shoes. Attachment of the cleats place the foot in a dorsiflexed position throughout the stance phase of running which produces and increases pressure upon the calcaneus. This creates a pull from soft tissue attachments such as plantar fascia which further leads to disorders like plantar fasciitis which needs to be treated early and by more advanced techniques to prevent further overuse injuries.

Objective: To find the effect of conventional physiotherapy exercises versus kinesiotaping in football players with plantar fasciitis.

Material and Method: In this pre-post intervention study 40 football players having plantar fasciitis were included. They were randomly divided into 2 groups with 20 individuals each. Group A was treated with conventional exercises and Group B with kinesiotaping along with conventional exercises for two weeks. After pre-post assessment, data was analysed with help of appropriate statistical methods.

Results: According to the result, in Group A and Group B there is significant difference in pain and measures of foot function index with p value < 0.0001.

Conclusion: The results obtained by studying both the groups in this study suggests that in treatment of plantar fasciitis, Conventional Therapy along with Kinesiotaping is more effective than Conventional Therapy alone.

Keywords: Plantar Fasciitis, Conventional physiotherapy exercises, Kinesiotaping, Pain assessment, Foot Function Index.

Introduction

Heel pain or pain in the sole of the foot is the most common site of pain in general population.1 It can occur due to tendinitis, disease of calcaneum, bursitis or inflammation of fat pad.1 It also commonly occurs due to plantar fasciitis.

Plantar fascia is a dense, fibrous connective tissue structure originating from the medial tuberosity of the calcaneus.1 There are 3 portions – medial, lateral and central bands of the fascia of which the central band is the largest portion. The fascia extends through the medial longitudinal arch into individual bundles and...
Plantar fascia is an important static support for the longitudinal arch of the foot. It acts as a shock absorber as it has an ability to elongate with increased loads.

Plantar fasciitis is a disorder in which there is non-inflammatory structural breakdown of the plantar fascia. In this disorder, there is classic presentation of gradual, insidious onset of inferomedial heel pain at the insertion of the plantar fascia. Typically, this pain is described as “burning”, “aching” and occasionally, “lancinating”. Pain and stiffness are worse with rising in the morning or after prolonged ambulation. Pain and tenderness are sometimes extended into the medial arch. Pain related to this condition may cause substantial disabilities and poor health-related quality of life.

It occurs due to obesity, direct repetitive microtrauma with heel strike, excessive running, standing on hard surfaces for prolonged periods of time, high arches of the feet, presence of limb length discrepancy or flat feet. It is more common in sports that involve running and long-distance walking. It is also common in non-athletic individuals who are overweight and obese.

There is 21.7% of prevalence rate of plantar fasciitis in individuals who play sports, especially in football players who use studs/cleats shoes.

Studs or cleats are protrusions on the sole or an external attachment to a shoe, that provide additional traction on a soft or slippery surface. They can be conical or blade-like in shape and made of plastic, rubber or metal.

There are three main types of football boots: round, hard ground and bladed. These cleats are often permanently attached to the shoe surface and sometimes they can be removed. The studs/cleats are mostly attached on the forefoot and heel surface of the sole leaving the midfoot area.

Attachments of these studs/cleats can create a negative effect on the sole of the foot. Attachment of the cleats place the foot in a dorsiflexed position throughout the stance phase of running which produces and increases pressure upon the calcaneus. This creates a pull from soft tissue attachments such as Tendo-achillies and plantar fascia which further leads to disorders like plantar fasciitis.

Considering that such a large number of professional football players are suffering from this condition it can be safe to assume that greater number of recreational players will be suffering from this condition as well. The fact that they do not have much knowledge about various types of shoes and how to wear them correctly will add to this problem. Plantar fasciitis has worse effects on the foot function, gait pattern, walking speed and even normal stance. There are several ways to assess foot pain and foot function and Foot Function Index respectively.

In this study foot function was assessed by using Foot Function Index. It is a simple yet reliable test. Test-retest reliability of the Foot Function Index total and subscale scores range from 0.87 to 0.69, while internal consistency ranged from 0.96 to 0.73. It is reported that the FFI is a reliable instrument for use in foot orthopedic intervention trials.

The Foot Function Index has been validated and determined to be a reliable instrument for patients with non-traumatic foot or ankle problems.

Also, recreational players are more prone to get plantar fasciitis as they do not have that much access as professionals does for such facilities. Often players ignore such heel pain thinking it will subside with time but then it leads to various other problems related to foot.

As we know that there is a conventional therapy used widely and for a long time to treat plantar fasciitis. But now there is need for more advanced therapeutic techniques to treat plantar fasciitis which will help in reducing the recovery period and can give more better results than the conventional therapy. And the advanced therapeutic technique for treating plantar fasciitis is Kinesiotaping.

Kinesiology taping or kinesiotaping is a curative tool and has been showing great results when used in treating variety of conditions. Taping has been used for a long time for the prevention and treatment of sporting injuries. It is a Japanese technique used with the intention to alleviate pain and improve the healing in soft tissues.

There are also other benefits of taping such as proprioceptive facilitation, reduced muscle fatigue, muscle facilitation, reduced delayed onset muscle soreness, pain inhibition, enhanced healing, such as reducing edema and improvement of lymphatic drainage
and blood flow.7

It can be applied in various shapes and sizes according to the needs of the condition.7 The shape selection depends upon the size of the affected muscle and the results to be achieved. Here, for plantar fasciitis “I” or “Fan” strip method can be used in the treatment which has a supportive or inhibitory effect on the plantar fascia.

Thus, recreational players need treatment and awareness of such therapeutic techniques for conditions which can lead to many disorders.

Methodology

The ethical clearance was taken from ethical committee of Krishna institute of medical sciences, Karad. There were 40 participants in the study. The study was taken place in the Physiotherapy department, Krishna Hospital. Treatment protocol was of 2 weeks. The subjects were assessed for foot function and foot pain at first. The type of study was experimental study. The study design was pre and post.

Procedure:

All the subjects were selected for the study according to the selection criteria. Demographic data and consent were taken from them. Included participants were divided in 2 groups by simple random sampling method. Pre and post assessment were taken before and after 2 weeks of the treatment respectively with the help of outcome measures.

Group A: In first week, Ultrasound therapy with pulsed mode and intensity of 0.8W/cm² for 7 minutes, for 5 days/week.

Contrast bath with hot water for 3 minutes and cold water for 1 minute for 5 times/session/day.

Active stretching of soleus and gastrocnemius muscles and plantar facia, 30 seconds hold for 5 times/day session.

In second week, Contrast bath with hot water for 3 minutes and cold water for 1 minute for 5 times/session/day. Active stretching of soleus and gastrocnemius muscles and plantar facia, 30 seconds hold each one, for 5 times/day session.

Strengthening exercises like toe curls with towel, 15 repetitions for 3 times.

Heel raise with towel, 15 repetitions for 3 times.

Marble pick-ups, 10 pick-ups for 2 times. All exercises were performed for 1 session/day.

Group B: In first week, Taping for 2 times/week. Patient position- prone lying. Fan method. Ultrasound therapy with pulsed mode and intensity of 0.8W/cm² for 7 minutes and for 5 days/week.

Contrast bath with hot water for 3 minutes and cold water for 1 minute for 5 times/session/day.

Active stretching of soleus and gastrocnemius muscles and plantar facia, 30 seconds hold each one, for 5 times/day session.

In second week, Contrast bath with hot water for 3 minutes and cold water for 1 minute for 5 times/session/day. Active stretching of soleus and gastrocnemius muscles and plantar facia, 30 seconds hold each one, for 5 times/day session.

Strengthening exercises like toe curls with towel, 15 repetitions for 3 times. Heel raise with towel, 15 repetitions for 3 times. Marble pick-ups, 10 pick-ups for 2 times. All exercises were performed for 1 session/day.
Results

1. Age wise distribution:

Table no. 1: Age wise distribution in study.

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Group A</th>
<th>Group B</th>
<th>Total</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Players</td>
<td>Percentage</td>
<td>Mean SD</td>
<td>Total</td>
</tr>
<tr>
<td>18-24</td>
<td>18</td>
<td>90%</td>
<td>21.39±1.79</td>
<td>20</td>
</tr>
<tr>
<td>25-30</td>
<td>02</td>
<td>10%</td>
<td>25.5±0.71</td>
<td>02</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>100%</td>
<td>21.8±2.11</td>
<td>20</td>
</tr>
</tbody>
</table>

Interpretation: Above table represents age wise distribution of the study. Out of 40 subjects, 90% subjects were from the age group 18-24 and 10% subjects were from the age group 25-30, in both A and B groups.

2. Pain score:

Table no. 2: Mean Pain Score.

<table>
<thead>
<tr>
<th>Pain Score(VAS)</th>
<th>Mean SD</th>
<th>Pre-Intervention</th>
<th>Post- Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>6.4±1.05</td>
<td>5.15±1.39</td>
<td>2.70±0.66</td>
</tr>
<tr>
<td>Group B</td>
<td>6.65±0.93</td>
<td>2.70±0.66</td>
<td></td>
</tr>
</tbody>
</table>

Interpretation: Above table represents mean pain score of pre and post intervention of subjects from both the groups.

Table no. 3: Paired and Unpaired ‘t’ test results.

<table>
<thead>
<tr>
<th>Pain Score</th>
<th>Paired t- test</th>
<th>Unpaired t- test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t- value</td>
<td>p- value</td>
</tr>
<tr>
<td>Group A</td>
<td>6.571</td>
<td>&lt;0.0001 (ES)</td>
</tr>
<tr>
<td>Group B</td>
<td>23.269</td>
<td>&lt;0.0001 (ES)</td>
</tr>
</tbody>
</table>
Interpretation: Above table represents results of paired and unpaired ‘t’ test of mean pain score of subjects from both the groups.

3. Foot Function Index Score according to Subscales:

Table no. 4: Mean scores of FFI.

<table>
<thead>
<tr>
<th>FFI</th>
<th>Mean SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A</td>
</tr>
<tr>
<td>FPS</td>
<td>16.80±1.70</td>
</tr>
<tr>
<td>FDS</td>
<td>28.20±1.36</td>
</tr>
<tr>
<td>ALS</td>
<td>5.10±0.31</td>
</tr>
</tbody>
</table>

Interpretation: Above table represents mean score of FFI according to its subscales of subjects from both the groups.

4. Foot Function Index Score:

Table no. 5: Mean scores of Foot Function Index.

<table>
<thead>
<tr>
<th>FFI</th>
<th>Mean SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-Intervention</td>
</tr>
<tr>
<td>Group A</td>
<td>53.53±3.61</td>
</tr>
<tr>
<td>Group B</td>
<td>29.47±2.12</td>
</tr>
</tbody>
</table>

Interpretation: Above table represents mean of total score of FFI of subjects from both the groups.

Table no. 6: Paired and Unpaired ‘t’ test results.

<table>
<thead>
<tr>
<th>FFI</th>
<th>Paired t- test</th>
<th>Unpaired t- test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t-value</td>
<td>p-value</td>
</tr>
<tr>
<td>Group A</td>
<td>49.069</td>
<td>&lt;0.0001 (ES)</td>
</tr>
<tr>
<td>Group B</td>
<td>49.093</td>
<td>&lt;0.0001 (ES)</td>
</tr>
</tbody>
</table>

Interpretation: Above table represents results of paired and unpaired ‘t’ test of mean score of total score of FFI of subjects from both the groups.
Discussion

A study reported that there is 21.7% of prevalence rate of plantar fasciitis in individuals who play sports, especially in football players who use studs/cleats shoes.

Thus recreational players need appropriate treatment and awareness of such therapeutic techniques like Kinesiotaping and proper strengthening exercises are necessary for preventing further complications.

In this study, 40 individuals who played football for recreation (all male) were taken. They were equally divided into two groups i.e. Group A and Group B. Individuals in the age group of 18-30 years were included in this study, out of which 18 (90%) individuals were in the (18-24) age group and 2 (10%) individuals were in the (25-30) age group, in both the groups. This age group consisted of those who played recreational football and did not have any training or experience in professional football.

In Group A, the subjects were given only conventional therapy for plantar fasciitis for two weeks and in Group B, the subjects were given conventional therapy along with Kinesiotaping. Pain score according to Visual Analogue Scale and Foot Function Index score of subjects of both the groups were taken before the treatment/intervention was given. The mean pain score measurements for Group A prior to the intervention was 6.4 and post intervention was 5.15 and for Group B, mean score pre-intervention was 6.65 and post-intervention was 2.70.

Statistical analysis of the pain score of pre and post intervention of both the groups was done to confirm that the difference between the pre and post measurements is significant. The comparison for pre and post pain scores was found to have a p-value of (<0.0001) which was very significant.

To analyze the impact of foot pathology on function in terms of pain, disability and activity restriction of foot was done by using the Foot Function Index (FFI). It is divided into 3 subscales viz; Foot Pain subscale (FPS), Foot Disability subscale (FDS) and Activity Limitation subscale (ALS) which consists of 23 items. Same as Pain score, measures of FFI were taken before and after the treatment. The mean score of FFI for Group A according to the FPS was 16.80, FDS was 28.20 and ALS was 5.10, and for Group B, FPS was 14.20, FDS was 25.20 and ALS was 5.00. Here, the scores of FDS was found to be more among the three subscales.

The mean scores of pre and post intervention of FFI for Group A were 53.53 and 31.49 and Group B were 29.47 and 17.34. Statistical analysis was done to compare the mean post intervention scores of Foot Function Index to confirm whether they were significant or not and it was found that the p-value for Group A was (<0.0001) which was extremely significant and for Group B the p-value was (<0.0001) which was extremely significant.

Analysis was done to compare the total scores of Foot Function Index post intervention of both the groups which were found to have a p-value of (<0.0001) which was extremely significant for both the groups.

Thus the results obtained by studying both the groups in this study suggests that in treatment of plantar fasciitis, Conventional Therapy along with Kinesiotaping is more effective than Conventional Therapy alone.

Acknowledgement: I sincerely thank the management of KIMSDU for allowing me to conduct this study by providing me the necessary requirements. I thank my Dean Dr. G. Varadharajulu sir for his support and guidance. I convey sincere thanks to my guides Dr. Khushboo Chotai and Dr. Amrutkuvar Rayjade for helping me in my research. I take this opportunity to thank all those who have been directly or indirectly involved for smooth conduction of my study.

Conclusion

On the basis of the result of the study it is concluded that in treatment of plantar fasciitis, Conventional Therapy along with Kinesiotaping is more effective than Conventional Therapy alone.

Source of Funding: Source of Funding: Krishna Institute of Medical Sciences Deemed to be University, Karad.

Conflict of Interest: There were no conflicts of interest in my study.

Ethical Clearance: The Institutional Ethical Committee has hereby given permission to initiate the research project titled, “Effectiveness Of Conventional Physiotherapy Exercises Versus Kinesiotaping In Recreational Football Players With Plantar Fasciitis”
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11. Windlass test: https://www.physio-pedia.com/Windlass_test


Metanalysis of Qualitative and Quantitative improvement in Active Rehabilitation of Post ACL Repair or Reconstruction

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Abstract

Background: Physiotherapy regimen is important in both Pre-Surgery and Post-Surgery phases. However, despite its widespread knowledge of importance of physiotherapy in both pre and post operation phases and during conventional therapy, the efficacy of early active intervention of physiotherapy in post-operative ACL repair/reconstruction is limited on the basis of quantitative and qualitative analysis.

Purpose: The aim of this meta-analysis was to analyze the randomized controlled trials having quantitative and qualitative improvement in parameters after active rehabilitation in subjects with post-repair/reconstruction of Anterior Cruciate Ligament.

Methods: The authors searched Google Scholar and PubMed from 2014 to 2019. Only downloadable randomized controlled trials were included in the study.

Main results: Twelve trials were included in the study with. The schedule of treatments varied greatly from certain days to 6 weeks or even follow up of 12 weeks. Subgroup analysis performed on Qualitative and quantitative assessment of pre-operative and post-operative active physiotherapy regimen showed significant statistical difference.

Conclusion: The result of the present study shows strong evidence to support the active physiotherapeutic treatment pre-operative as well as post-operative in subjects with ACL repair/reconstruction, which helps in improving both qualitative and quantitative parameters.

Key words: Meta-analysis, Qualitative, Quantitative parameters, Active rehabilitation, ACL repair, reconstruction

Introduction

Anterior Cruciate Ligament injury is common in knee joint injuries. Maximum injury of ACL occurs at sudden twisting and turning of flexed knee at the range 30 degrees. According to grading system given by Hopkin’s Usually grade III: is opted for surgical reconstruction and grade I and II: for repair/conservative management.

Most of the musculoskeletal operations require both pre- and post- Physiotherapy regimen. Pre-operative physiotherapy regimen helps in easy future rehabilitation of the subject as the surgery causes definite reduction of the strength within the local muscles. Post-operative physiotherapy regimen helps in increasing the strength as well as range of motion, reducing pain, early recouping of the daily activities, and functionally improving the subject. Various recent therapies used during Physiotherapy pre-operative and post-operative regimen which are known to be significant are: Whole body vibration, cross education, eccentric cycling, Delaware-Oslo, MOON, FES, Jump training with Body weight support.

There are very few randomised controlled trials defining both the qualitative and quantitative improvement in post-operative ACL. Further, information about qualitative and quantitative assessment of the change in parameters with active rehabilitation of ACL post-repair/reconstruction is limited. Hence it is necessary to know the improvement both qualitatively and quantitatively in cases with post-ACL repair/reconstruction after active rehabilitation. Hence this meta analysis is undertaken.
Method

Literature Search:

The reviewer searched the following computerized bibliographic English language databases: Google Scholar and PubMed from 2014 to 2019. The highly sensitive Cochrane collaboration strategy was used which targeted only randomized controlled trails.

Literature selection:

Studies were included if they met inclusion criteria and excluded if they didn’t. Inclusion criteria: 1) Randomised Controlled trial of Physiotherapy following post-ACL reconstruction/repair, 2) RCT’s having Immediate physiotherapy referral, 3) RCT’s having Active physiotherapy intervention, 4) RCT’s having EMG analysis of quadriceps, hamstring, gastrocnemius (any or all of the muscles) or any other qualitative analysis, 5) RCT’s having any one of the disability scales as quantitative analysis relating to knee and 6) RCT’s which can be downloaded 7) RCT’s published after 2014 Exclusion Criteria: 1) Inclusion of any other trauma except ACL injury, 2) Irregular physiotherapeutic regimen, 3) Passive physiotherapeutic regimen, 4) Bilateral ACL injury.

Quality assessment:

Quality of the studies recruited in this study was assessed by quality list from Cochrane Back Review Group18. Data were extracted Independently and checked for accuracy for the purpose of methodological quality.

Ethical Clearance: Ethical clearance obtained from institutional ethical committee Krishna Institute of Medical Sciences Deemed to Be University Karad.

Statistical Analysis

Randomized controlled trials in this study are classified by the year of publication, type of active rehabilitation, follow up time, outcome measure and improvement seen or not. Effect size was calculated of the changes in outcome measure between the control group and active rehabilitation part of the experimental group of various studies. Also, comparison was done between active therapy of each study to analyze the most effective therapeutic approach improving both qualitative and quantitative parameters post ACL repair/ reconstruction using SPSS version 16.0.

Results

Search strategy identified 1250 potential abstracts among which 150 randomized controlled trials were extracted out of which only 12 fit into the criteria of the study- Appendix 1. Table 2 depicts the key clinical and methodological characteristics of each included study.

Evidences:

1. Whole body vibration exercise protocol versus a standard exercise protocol after ACL Reconstruction: A clinical Randomized Controlled Trial with Short Term Follow-up by Berschin G. et al; 2014; quality score=5.1.1. Sample size was 20 in each group with outcome measure: Isometric and isokinetic muscle strength measurements, Lysholm score, neuromuscular functions. Improvement was seen in group with whole body vibration.6

2. Effects of early whole body vibration treatment on knee neuromuscular function and postural control after anterior Cruciate ligament reconstruction: a randomized controlled trial by Pistone et al;2016; Quality score=4.2.2. Sample size was 17 in each group. Pre physiotherapy intervention included whole body vibration therapy. Outcome measure were Maximum voluntary isometric strength during knee flexion and extension and Balance on force platform. Improvement was seen in whole body vibration technique at one month itself.7

3. Effect of targeted exercise on knee-muscle function in patients with persistent hamstring deficiency following ACL reconstruction- study protocol for a randomized controlled trial by Bregenhof; 2018; quality score= 6.2.2. 25 sample was included in each group with Maximal isometric knee flexor-extensor strength, KOOS, IKDC, Tegner activity score Rate of force development for knee flexion and extension, tendon regeneration and potential muscle hypertrophy evaluated by MRI, Postural control, kinetic/kinematic gait characteristics and knee related functional capacity. None of the result were mentioned.8

4. Cross education does not accelerate the rehabilitation of neuromuscular functions after ACL reconstruction: a randomized controlled trial by Zult et al; 2018; quality score=7.3.4. 22 sample was included in each group, with outcome measure: Isometric quadriceps maximal voluntary contraction, Voluntary quadriceps activation, quadriceps force accuracy and variability,
knee joint proprioception and single leg balance and improvement was seen in standard treatment protocol and cross education didn’t show any improvement.9

5. A comparison between Modified Robert Jones Bandage and Intermittent Cold Pack in Arthroscopic Anterior Cruciate Ligament Reconstruction: A Prospective Randomized Controlled Trial by Kijkunasathian et al; 2017; quality score=3.2.2. sample size was 19 in each group with outcome measures were Functional outcomes: positive quadriceps set, active straight leg raise, active knee flexion and limited knee extension, Knee swelling and Overall patient satisfaction. Improvement was seen in intermittent cold packages.10

6. Eccentric cycling rehabilitation in anterior cruciate ligament reconstruction: a randomised controlled trial of strength and biomechanical outcomes by Milandri G. et al; 2017; quality score=1.1.1. outcome measure were: Maximal load rate, Initial load rate, Average load rate, Impact force, Hip-knee angle during gait and hip- knee moments and Frontal knee valgus angle. Improvement in all qualitative parameters except average loading rate and impact force.11

7. Comparison of ACL-SPORTS Randomized Controlled Trial With Delaware-Oslo and MOON Cohorts by Capin et al; 2019 with quality score= 5.2.4. sample size was 20 per group. Outcome measures were Quadriiceps strength, Hop test, Functional outcomes (IKDC, KOOS pain, KOOS symptoms, KOOS ADL, KOOS Sports, KOOS QoL) Return to sport rates. Addition of perturbation did not add any benefit to the therapy.12

8. Pain experience and functional outcome of inpatient versus outpatient anterior Cruciate ligament reconstruction, an equivalence randomized controlled trial with 12 months follow up by Valkering et al;2015; quality score=6.2.2. Sample size was 23 per group with outcome measure: Functional outcomes: Lysholm, Tegner and IKDC Other: Pain experience and readmission rate. No significant difference were found between groups.13

9. Functional electrical stimulation following anterior cruciate ligament reconstruction: a randomized controlled pilot study by moran et al; 2019; quality score= 3.2.2. Sample size was 10 per group with outcome measures were Gait speed, single limb stance gait symmetry, Quadriceps isometric peak strength ration, peak strength inter-limb symmetry. FES was more better option to improve outcome measure.14

10. Kinesiotaping as a treatment method in the acute phase of ACL reconstruction: A double-blind, placebo-controlled study by Balki et al; 2016; quality score= 7.3.4. Sample size was 15 in each group with outcome measure: Lysholm, modified Cincinatti and Tegner scores on first and third post-operative months. KT therapy showed better improvement in all the outcome measures.15

11. A Randomized Control Trial of Acute Post Operative Care Following Anterior Cruciate Ligament Reconstruction: A Comparison of Two Protocols by Hallworth W. et al; 2014; quality score= 3.2.2. sample size were different in both groups i.e. 20 in one group and 17 in another. Outcome measures were: International Knee Documentation Committee (IKDC) Subject Knee Evaluation Form, Numeric Rating Scale for Pain (NRSP), Circumferential measure, Range Of Motion and improvement was shown significant in group given with DVD for performance of exercise.16

12. Clinical Efficacy of Jump Training Augmented With Body Weight Support After ACL Reconstruction: A randomized controlled trial by Elias et al; 2016; quality score= 6.3.3. Sample size was 15 in each group with outcome measure= International Knee Documentation Committee (IKDC) questionnaire, leg landing mechanics via motion analysis, knee joint effusion using a stroke test, surface electromyography–generated co-contraction index during a single-legged landing. Jump training with augmented body weight showed good improvement than without.17

The studies included 2458 patients; study sample size ranged from 10 to 409. Quality varied but tended to be higher in recent studies.

Active physiotherapeutic regimen versus improvement in outcome measure

Forest plots for qualitative measures and quantitative measures:

Effectiveness of various Active Physical therapy on Quantitative measure:
Compared with sham therapy, patients receiving treatment that included various Active Physical therapy intervention were definitely better except for one study which showed no better improvement than control group. On analyzing all the studies the maximum improvement was seen in disability index which one of the inclusion criteria of the study with 95% CI. Most significant effect was seen in study by Capin et al\textsuperscript{12} and Hallworth et al\textsuperscript{16} who used therapies like ACL SPORT- Protocol and A standardized protocol with DVD given to subjects respectively. Kinetic gait parameter was significant in the study by Elias et al including vertical ground reaction force as well by Moran U et al. Present showed significant effectiveness in quantitative parameters like kinematic gait parameters and maximum activation of quadriceps and hamstring by Zult et al\textsuperscript{9} and Elias et al\textsuperscript{17} and with p value 0.015 and 0.001 respectively.

Effectiveness of various Active Physical therapy on Qualitative measure:

Most significant effect was seen in study by Zult et al\textsuperscript{9} and Elias et al\textsuperscript{17} who used therapies like standardized treatment regimen and Jump training with body weight support respectively. Isometric maximal voluntary muscle contraction balance control and muscle strength of hamstring and quadriceps were also highly significant. The present study showed significant effectiveness in quantitative parameters like kinematic gait parameters and maximum activation of quadriceps and hamstring by Zult et al\textsuperscript{9} and Elias et al\textsuperscript{17} and with p value 0.015 and 0.001 respectively.

**Discussion**

All the therapies provided maximum benefit in improvement of both qualitative and quantitative parameters in subjects with post-ACL repair or reconstruction after Active physiotherapy regimen. Our comparison of various active physiotherapy regimen with control group stated that various physiotherapy protocols act differently on different parameters, which are both qualitative as well as quantitative. Most significant protocols were given in the studies by Capin
et al\textsuperscript{12}, Elias et al\textsuperscript{17} and Zult et al\textsuperscript{9}. Capin et al; the study compared three different protocol with one control group that is pure training of strength, agility, plyometric and secondary prevention, when compared to SAPP + perturbation training showed no statistical significance, but on comparison of this control group to MOON and Delaware-Oslo cohort study; ACL-SPORT [ACL-Return to SPORT protocol] group that is the control group showed more significant changes.\textsuperscript{12}

Elias et al; jump training is very important part of rehabilitation of subjects with ACL repair or reconstruction, because it activates the muscle in sudden weight bearing position, and also during landing on uneven surfaces. But, jump training done directly would harm the operative procedure done on the fresh graft of ACL. The graft of ACL is fixed with screws which on sudden landing me cause damage. Also, ACL operated subjects are more prone to early development of osteoarthritis, hence usage of body weight support system will definitely help the rehabilitation process. Initially weight bearing exercises and walking are very painful to the subjects who have undergone ACL repair or reconstruction. Body weight support system, grades the amount of weight borne by the individual while performing weight bearing exercises and also during walking. Hence in this study both qualitative as well as quantitative parameters were seen to improve. Vertical jump training is specifically given to improve the co-contraction of musculature along the knee; both quadriceps as well as hamstrings. But as the loading is painful in knee disorders and vertical jump being dynamic loading if done in ACL injured cases which is therapy followed for attaining dynamic stability in the knee between 6-48 months.\textsuperscript{17}

Zult et al; the study stipulated that the usage of cross education is not beneficial in the regimen of ACL repair/reconstruction. The study compared two groups, one with cross education and one without. At the end both of them were equally significant hence, between group significance was not seen. Though, it is known that training the good leg in any of the operated cases, in lower extremity was helpful in early weight bearing activities like walking and transfer, this study had a negative impact on that and remarkably it had a negative effect of central activation ratio of quadriceps which was calculated and also decreased limb symmetry index of quadriceps muscle strength by the end of the session. Hence, cross education wouldn’t be beneficial in treatment regimen followed for treating subjects who have undergone ACL reconstruction or repair.\textsuperscript{9}

**Conclusion**

Every active therapy deals with improvement of various outcome measures. This meta analysis have gathered twelve such randomised controlled trials passing the quality test by Cochrane. On statistical analysis the significant improvement among all the studies with respect to disability scale, strength training and muscle performance was ACL- Return to SPORT Protocol along with closed kinetic training by jump training along with augmented body weight support system.

**Summary**

This study will used randomized controlled design to investigate the effect active physiotherapy rehabilitation on qualitative and quantitative parameters in subjects with ACL repair or reconstruction compared with controls, on knee-joint function in. The trial results helped to determine which active protocol or active physiotherapy regimen helped in improving both qualitative as well as quantitative parameters.

**Source of Funding:** Self

**Conflicts of Interest:** None

**References**

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Effectiveness of Lifestyle Modification in Late Adolescent Females with Normal BMI Polycystic Ovarian Syndrome

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Abstract

Background and Objectives: The prevalence of PCOS varies from 2.2% to 26% in different countries. Between 20-50% of women with PCOS are normal weight or lean and the pathophysiology of the disorder in these women may differ from that of obese women; It has been suggested that PCOS develops in non obese women because of a hypothalamic pituitary defect that results in increase release of LH. To determine the effectiveness of life style modification in late adolescent with normal BMI polycystic ovarian syndrome.

Material and Methodology: 28 Normal BMI women with polycystic ovary syndrome were selected for the study. They received the life style modification including diet plan exercise plan. Pre –assessment of body weight is measured by body mass index (BMI),waist-hip ratio(W-H Ratio) and quality of life was scored as per polycystic ovarian syndrome questionnaire(PCOSQ) and post-interventional assessment was taken for the same after 3 months. The exclusion criteria included Type II diabetes, Cardio vascular problems, any concurrent hormone therapy within 6 weeks, pregnant women’s or who are willing to conceive.

Result: Statistical analysis for PCOSQ was extremely significant (P value=0.0001).Statistical analysis for BMI revealed not significant in post intervention .Statistical analysis for W-H Ratio revealed not significant in post intervention.

Conclusion: Lifestyle modification helped in maintaining weight and showed increased quality of life in women with polycystic ovarian syndrome (PCOS).

Key Words: Life style modification, normal body mass index, walking, Polycystic ovarian syndrome.

Introduction

Polycystic ovarian syndrome (PCOS) has been defined by the national institute of health and Rotterdam criteria as a hormonal disorder characterized by the presence of at least one polycystic ovary (presence of multiple cysts) accompanied by ovulatory dysfunction and excessive secretion of androgens.[15] PCOS is a fairly common condition in women of reproductive age[1].Polycystic ovarian syndrome is heterogeneous. [1] and nowadays it is recognized as most common endocrinopathy in reproductive aged women having key features like menstrual irregularity, elevated androgens and polycystic appearing ovaries.[2]

The prevalence of PCOS varies from 2.2% to 26% in different countries.[4] women with PCOS either have normal weight or are found lean in 20-50% women and the pathophysiology in these women may differ from that of obese women; it has been suggested that PCOS develops in non obese women because of a hypothalamic pituitary defect that results in increase release of LH.[4]
The prevalence of normal weight and underweight patients with polycystic ovarian syndrome been reported 1.5-6.6%.[7] The prevalence of PCOS is 9.13% in Indian adolescents.[15] The prevalence rate 6% in South India discover that the probability of urban women vulnerable to acquiring PCOS are 0.1 times higher than women in rural India.[16]

In previous study the subjects taken between the age of 18 to 24 years.[16]

Even in lean women with polycystic ovarian syndrome, we can see the higher waist-hip ratio, greater intra peritoneal and visceral fat.[7] By definition women with BMI< 25 are non-obese and with BMI >25 considered obese.[7]

Polycystic ovarian syndrome is associated so strongly with obesity therefore the women with normal weight often go undiagnosed.[10] Polycystic ovarian syndrome requires at least two of the following characteristics for the diagnosis; clinical or biochemical hyperandrogenism, anovulatory menstrual dysfunction and polycystic ovarian on ultrasound.[9]

Young adolescent girls experiences symptoms from irregular menses, amenorrhea, ovarian cyst, menorrhagia, hirsutism , acne, skin pigmentation. Symptoms like anxiety, depression, thyroid problem also may include.[5]

Obesity, type 2 diabetes, dyslipidemia, hypertension and cardiovascular disease has been linked with PCOS.[11] PCOS is an emerging health problem during adolescence therefore promotion of healthy lifestyle and early intervention are required to prevent future morbidities.[6]

In normal BMI women the polycystic ovarian syndrome seen because the connection between the pituitary gland and the ovaries broken down.

**Importance of Lifestyle Modification**

Life style modification commonly used as the first line treatment for PCOS women.[8] Life style modification contains dietary modification, physical activity and behavioral changes.[8] Exercise is important component of life style intervention by improving several benefits on cardio vascular, metabolic, reproductive and psychological health of women with PCOS.[3] For physical activity, participation and compliance feeling of pleasure during exercise is an important factor.[10] In respect of new randomized controlled trail, arranged and regular physical exercise can improve insulin sensitivity and menstrual regularity in PCOS women.[3]

Maintaining weight can help reduce diabetes risk.[10]

**METHODOLOGY**

Ethical clearance was obtained from the Institutional Ethical Committee, KIMS DHU, Karad. It is an experimental study was carried using cross sectional study design. The study was conducted in and around Karad, Maharashtra and was carried over a period of 12 week. This study include 28 women diagnosed with PCOS. The study protocol was approved by Regional Ethical Committee. Exclusion criteria included Type II diabetes, Cardiovascular problems, any concurrent hormone therapy within 6 weeks, pregnant women’s or who are willing to conceive. After baseline assessment, which include demographic data, body mass index (BMI), W-H ratio, questionnaire PCOS (PCOSQ) using random sampling method.

The hip and waist circumferences were determined using a tape measure, the widest circumference of buttocks and the smallest circumference of the hip.

The participant receive lifestyle modification which include diet, exercise, behavioral therapy

**Exercise**

Warm up exercise and cool down exercise for 10 minutes. The pcos exercise recommended atleast for 3 times per week. Aerobic exercise such as walking and jogging performed for 50-60 minutes per session.[13]

**Diet**

High protein diet include egg white, beans, pulses. Fruits like apple, guava, pear, grapes, oranges etc should be include. Tomatoes, sweet potato, pumpkin, almonds etc. High fiber and leafy vegetables are included. Avoid food with high glycemic index such as bakery and dairy products. Pulses plays an important role in weight control[12]

**Behavioural Management**

Motivation and cognitive behavioural therapy to avoid negative thoughts and to improve state of mind.
Statistical Analysis and Results

Statistical analysis of the recorded data was done by using the software SPSS version 2.0. Study design is cross sectional. Arithmetic mean and standard deviation was calculated for each outcome measure. Paired t test was done.

**BMI**

**Table no 1: Pre and post BMI score**

<table>
<thead>
<tr>
<th></th>
<th>MEAN+ SD</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRE</td>
<td>21.6+1.3</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>POST</td>
<td>21.7+ 1.5</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

**WAIST HIP RATIO**

**Table no 2: Pre and Post W-H Ratio**

<table>
<thead>
<tr>
<th></th>
<th>MEAN+ SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRE</td>
<td>0.84+0.04</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>POST</td>
<td>0.84+0.04</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

**PCOSQ**

<table>
<thead>
<tr>
<th>Emotion</th>
<th>Weight concern</th>
<th>Body hair</th>
<th>Menstrual irregularity</th>
<th>Infertility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean+ SD</td>
<td>Mean+ SD</td>
<td>Mean+ SD</td>
<td>Mean+ SD</td>
<td>Mean+ SD</td>
</tr>
<tr>
<td>PRE</td>
<td>30.64 +3.4</td>
<td>17.39 +2.0</td>
<td>17.89 +2.0</td>
<td>14.17 +2.4</td>
</tr>
<tr>
<td>POST</td>
<td>39.78 +3.15</td>
<td>28.14 +1.4</td>
<td>25.75 +1.93</td>
<td>20.64 +2.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>MEAN+ SD</th>
<th>P value</th>
<th>T value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRE</td>
<td>19.09 +6.4</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>POST</td>
<td>27.08 +7.2</td>
<td>&lt;0.0001</td>
<td>35.24</td>
</tr>
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</table>

**Table no 3: Pre and post PCOSQ**

<table>
<thead>
<tr>
<th></th>
<th>Mean+SD</th>
<th>Mean+SD</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POST</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>21.6+1.3</td>
<td>21.7+ 1.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>W-H Ratio</td>
<td>0.84+0.04</td>
<td>0.84+0.04</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PCOSQ</td>
<td>19.09 +6.4</td>
<td>27.08 +7.2</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Discussion

Polycystic ovarian syndrome is associated so strongly with obesity therefore the women with normal weight often go undiagnosed. Even in lean women with polycystic ovarian syndrome, we can see the higher waist-hip ratio, greater intra peritoneal and visceral fat.

Exercises and accepting healthy lifestyle created a good body and mental image in the patient that drastically improved which directly showed impact on higher quality of life according to PCOSQ and also there is no marked significance in other outcome measures.

Visceral fat which is seen in normal BMI women’s, the visceral fat itself secretes adipokines which impair insulin sensitivity in tissue such as liver and skeletal muscle, insulin resistance is manifested as decrease in glucose transport and decline in muscle glycogen synthesis in response to circulating insulin. In normal BMI women the polycystic ovarian syndrome seen because the connection between the pituitary gland and the ovaries broken down.

Because of the lifestyle modification which include exercise, diet the connection between the pituitary gland and the ovaries reestablished which is helpful for to regulate the menstrual cycle.

In the previous study in polycystic ovary syndrome and weight management the conclusion was prevention from weight gain in normal BMI was crucial component.

And in benefits of short-term structured exercise in non-overweight women with polycystic ovary syndrome: a prospective randomized controlled study the result was after 8 weeks of exercise the gap between the menstrual cycle became shorter and the p value <0.05 which considered as significant.

The participant was given lifestyle modification which included exercise with diet and behavioral management which added plus point in keeping them motivated towards their body image and keep holding their diet by setting different goals and target.

The PCOSQ score is the important outcome measure the p value(<0.0001) which was extremely significant and the paired t test performed the t value was(35.24). BMI was slightly change in the some women’s.

Women felt challenging to continue diet and exercises in their routine, which is further challenge to keep going with gained weight. Hence, continuing with lifestyle modification is a challenge and should be kept as long term follow-up, to have good effect.

Conclusion

This study concluded that lifestyle modification for normal BMI PCOS women was effective in maintaining weight and quality of life. The quality of life was improved according to PCOSQ. There by we conclude that lifestyle modification should be the first line treatment for PCOS women.

Conflict of Interest: The authors declare that there is no conflict of interest.

Acknowledement: The authors would like to express their special gratitude towards all the women’s that participated in the study. We would also like to thank our families and institution for their everlasting support which enabled us to continue our research activities.

Ethical Clearance: This study has undergone ethical clearance through the university level ethical committee of Krishna Institute of Medical sciences, Deemed to be University, Karad.

Source of Funding: No funding

Abbreviation:
BMI-Body mass index
W-H Ratio-waist hip ratio
PCOSQ- Polycystic ovarian syndrome questionnaire

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Study of Profile of Sexually Related Unnatural Deaths

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Abstract

Sexual crimes against women are on the rise. It has been well documented that sexual assault cases are under reported. The study was conducted in the Department of Forensic Medicine, M.S. Ramaiah Medical College, Bangalore during the period from September 2008 to February 2010, a period of 18 months where in 52 cases were due to sexually related deaths.

Keywords: Unnatural Deaths, Sexual assault, Autopsy.

Introduction

Sex is a natural phenomenon and is necessary for the continuity of human race on this planet of earth and sexual exploitation is the worst form of degradation of those who indulge in it. The rising trend in the sexual offence cases is a reflection of the western influence in our society. Chastity is very important in the life of an Indian woman. Virginity is considered as an essential prerequisite for a girls to become a bride. The violation of virginity subjects a woman to considerable shame and humiliation. A study reveals, only about 10-50% of the female victims reported about their assaults. Incidence of sexual abuse and death particularly among young and adolescent girl is reported almost daily. It not only humiliates and makes a woman powerless, but for many, the effects are long lasting. The present study was conducted with the objectives to know the age, sex, marital status and socioeconomic status of the victim.

Materials and Method

The study was conducted in the Department of Forensic Medicine, M.S. Ramaiah Medical College, Bangalore during the period from September 2008 to February 2010, a period of 18 months. Out of 1062 unnatural deaths during the study period 52 cases were due to sexually related deaths. All the cases subjected for medico legal autopsy with alleged history of death being sexually related, when there is evidence or acknowledgments of sexual activity in the causation of death were studied. Detailed information regarding the circumstances of death was sought from the police, victim’s relatives, friends, and visit to the scene of occurrence or by the photographs of the scene of occurrence. Unnatural deaths due to sexual harassment, sexual offence, premarital affairs /sexual relationship based on circumstantial evidence and deaths due to sexual asphyxia are included in the study. Deaths due to sexually transmitted disease, natural disease and due to dowry related were excluded.

Results and Observation

In present study out of 1062 unnatural deaths, 52 cases were due to sexually related. Among them 43 cases were female (82.7%) and 09 cases were male (17.3%).

The most vulnerable age group is between 20-29 years (50%) of the total deaths followed by those in 10-19 years (21.17%). Least number of deaths in the age
group of ≥ 50 years (1.92%) followed by age group of 0-9 years (3.84%). The maximum number of victims (57.69%) were from the middle socioeconomic status followed by lower socioeconomic status (34.62%).

The maximum number of deaths were observed in married victims (50%), more so during the early period of their marriage and followed by unmarried victims (46.16%).

The maximum number of victims (57.69%) were from the middle socioeconomic status followed by lower socioeconomic status (34.62%).

Table no. 1: Distribution of sexually related unnatural deaths according to sex

<table>
<thead>
<tr>
<th>Sex</th>
<th>Count</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>09</td>
<td>17.3%</td>
</tr>
<tr>
<td>Female</td>
<td>43</td>
<td>82.7%</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table no. 2: Distribution of victims based on age

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Age group</th>
<th>Total</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0-9 years</td>
<td>02</td>
<td>3.84</td>
</tr>
<tr>
<td>2</td>
<td>10-19 years</td>
<td>11</td>
<td>21.17</td>
</tr>
<tr>
<td>3</td>
<td>20-29 years</td>
<td>26</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>30-39 years</td>
<td>09</td>
<td>17.3</td>
</tr>
<tr>
<td>5</td>
<td>40-49 years</td>
<td>03</td>
<td>5.77</td>
</tr>
<tr>
<td>6</td>
<td>≥50 Years</td>
<td>01</td>
<td>1.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td>52</td>
<td>100</td>
</tr>
</tbody>
</table>

Table No.3: Distribution of victims based on marital status

<table>
<thead>
<tr>
<th>SL.No.</th>
<th>Marital status</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>26</td>
<td>50.00</td>
</tr>
<tr>
<td>2</td>
<td>Un married</td>
<td>24</td>
<td>46.16</td>
</tr>
<tr>
<td>3</td>
<td>Not Known</td>
<td>2</td>
<td>3.84</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>52</td>
<td>100</td>
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</table>
Table No. 4: Distribution of victims based on socioeconomic status

<table>
<thead>
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<th>S-E Class</th>
<th>Number</th>
<th>Total</th>
<th>Percentage</th>
</tr>
</thead>
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<td>Upper</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>03</td>
<td>03</td>
<td>5.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>15</td>
<td>30</td>
<td>57.69</td>
</tr>
<tr>
<td>III</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>17</td>
<td>18</td>
<td>34.62</td>
</tr>
<tr>
<td>V</td>
<td>01</td>
<td>01</td>
<td>1.92</td>
</tr>
<tr>
<td>Not Known</td>
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<td>01</td>
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</tr>
<tr>
<td>Total</td>
<td>52</td>
<td></td>
<td>100</td>
</tr>
</tbody>
</table>

Discussion

The present study was conducted with the objectives to know the age, sex, marital status and socioeconomic status of the victim. Out of 52 unnatural sexually related deaths, 43 cases were female (82.7%) and 09 cases were male (17.3%). Females outnumbered the males which is quite understandable as in most of the cases the females are vulnerable on account of their guilable age, physical built as against the strong physique and androgenic behaviour of the dominant male. Similar findings were observed by J Mc Namara\(^3\) where most of them are females and were aged between 11 to 45 years.

The most vulnerable age group is between 20-29 years (50%) of the total deaths followed by those in 10-19 years (21.17%). As these age groups are sexually more active and hence are vulnerable. Least number of deaths in the age group of ≥ 50 years (1.92%) followed by age group of 0-9 years (3.84%). Similar findings were observed by Mc Cook A\(^4\) on African-American women, most of them were in the age group between 15 to 34 years.

The maximum number of victims (57.69%) were from the middle socioeconomic status followed by lower socioeconomic status (34.62%), as India is a developing country and much of the people fall in this group, also the changing social trends of nuclear families, unemployment, illiteracy, financial problems etc. Similar findings were observed by AK Srivastava\(^5\), where the most of the victims were belonged to middle or lower socioeconomic status.

The maximum number of deaths were observed in married victims (50%), more so during the early period of their marriage – extramarital affair was the main reason and followed by unmarried victims (46.16%). Similar findings were observed by AK Srivastava\(^5\), where more deaths were seen in newly married victims (86%).

Conclusion

Sexually related deaths constituted 4.8% of autopsies conducted. Maximum number of sexually related deaths occurred in the age group 20-29 years (50%) followed by 10-19 years (21.17%). Females constituted 82.70% of the victims. Most of the victims were married and were from middle socio economic status. The findings of this study may only be the tip of the iceberg as many sexually related deaths might never be detected, due to various reasons like cover up by the victims own family, improper investigation or because of false history furnished. An awareness must be created in the community to motivate the victims to report and more reporting centers must be opened to overcome the fear of victims.

Conflict of Interest: None.

Source of Funding: None.

Ethical Clearance: Obtained from the Institutional Ethics Committee.
References


Knowledge and Awareness about Medical Ethics among Medical Practitioners in a Teaching Medical College and Hospital, Chennai

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Abstract

Objective: The purpose of this study is to assess the knowledge and awareness towards medical ethics among medical practitioners. The results of this study may be used to guide the tutors of medical students, curricula designers and to bring change in attitude among the doctors towards the patient.

Methods: This descriptive, cross-sectional analytic study was conducted to assess the knowledge and awareness towards medical ethics among medical practitioners. A pretested semi-structured questionnaire was given and collected from the participants. After obtaining the data, it was entered in excel spreadsheet and was analysed using SPSS software and frequencies and percentages were obtained.

Results: Most of the participants have gained their knowledge on medical ethics from seminar and workshops (40%) and clinical training (36%) followed by other sources such as journal, lectures, ethical books etc., 71% of the participated medical practitioners agreed that “During treatment, the patient’s wishes must always be adhered to”. 66% of the participants disagreed that “Privacy of one patient may be ignored for the benefit of the larger group”.

Conclusion: This study highlights the lack of improvement in ethics awareness corresponding to increasing exposure to medical education in the last few years. Thus, with the help of the results obtained from this study, it can be used to devise means to sensitize them to these issues and appropriately training them.

Keywords: Ethics, Medical practitioners, Knowledge and awareness, Medical ethics.

Introduction

The moral principles which should guide the members of the medical profession in their dealings with each other, their patients and towards the state are dealt in medical ethics. These moral principles include respect for autonomy, non-maleficence, beneficence, and justice. Ethics reflects the conduct, character, and attitude of a doctor. There are several codes of conduct. The basic principles for medical professionals are discussed in hippocratic oath. At the time of registration of a medical graduate, he/she has to sign a declaration which is the modern version of hippocratic oath and is called as the Declaration of Geneva (1948).

Challenges of an ethical nature are abound in modern-day medicine. Patients, their families, those who provide medical care and institutions, where this care is conducted face difficult choices almost as a routine¹². Ethics is always an integral part of healthcare. Nowadays, modern technologies have blurred the line between medical ethics and quality care.

Ethical dilemmas are usually seen in areas such as abortion, contraception, treatment of patient with a terminal illness, professional misconduct, maintaining a patient’s confidentiality, the doctor’s professional relationship with patient’s relatives, religion, traditional medicine, and conflict of interests. Ethical violation
is professional misconduct and state medical council takes action. A physician shall report to appropriate authorities those physicians who practice unethically or incompetently or who engage in fraud or deception. It is very important to train the students about medical ethics, so that they are capable of solving any kind of ethical issues and to prevent themselves from ethical dilemmas during their practice. Every hospital must have an ethics committee to ensure that appropriate ethical values are being applied within hospitals.

There are varied views on strategizing the teaching of bioethics. The teaching of bioethics should also be holistic. Students could be taught about the value of the “heart” over the “mind” of the system of values and beliefs in a community, and of the need to understand the lived experiences of patients; while also incorporating various ethical approaches\(^9\).

In recent days, medical profession has been commercialized. Ethics teaching has a significant influence on the professionalism and moral qualities of medical professionals. In India, due to the cultural mosaic\(^10\), the teaching of bioethics needs to encompass the various perceptions of morality and ethics unique to people from different cultural, socioeconomic and geographical backgrounds\(^11\). The training in this subject should be integrated with the local social and cultural values.

Training in medical ethics has been made mandatory in the undergraduate curriculum by the regulatory body of medical education, Medical Council of India\(^7\). There are medical colleges in India with a standardized ethics curriculum, and with provisions for evaluation\(^8\). The first step in formulating an ethics curriculum may be to determine the level of the basic knowledge and attitudes among the medical practitioners. Few standard yardsticks have been designed to measure what is known and practiced so as to ensure that educational efforts are better targeted\(^13\).

Various methodologies have been tried to stimulate better ethical conduct in health professionals such as lectures, seminars, workshops etc. Against this background, this study is aimed to assess the level of awareness and knowledge regarding medical ethics among the study participants. It is a questionnaire based retrospective study.

**Methods and Materials**

The present cross-sectional retrospective study was conducted in Saveetha Medical College and Hospital, Chennai. Medical Practitioners were included in the study. A total of 100 medical practitioners were studied. Both male and female took part in this study. Most of the approached doctors were from both clinical and non-clinical departments of Saveetha Medical College and Hospital, Chennai. Even the postgraduates were included in this study. In this study, 126 doctors were approached, out of which 110 of them were willing to take part in this study. They were given the liberty to pull out of the study whenever they wanted if they felt unsafe or uncomfortable with the study. Out of 110 questionnaires, the 10 questionnaires which were partially filled or incomplete were excluded from the study. Only the questionnaires in which the consent form was filled properly were included in the study. The study period was from 21st January 2019 to 30th March 2019. Institutional ethics committee\(^\text{IEC}\) clearance was applied for and obtained. All the medical practitioners who took part in the study were briefed on the study’s purpose, and requested to participate in it. The questionnaires were distributed after describing the purpose of the study. The participants were assured that participation was voluntary, and confidentiality would be maintained. A pretested semi-structured questionnaire was devised, based on the previous research studies and in consultation with faculty members of the department of forensic and state medicine. Some questions were also derived from the code of medical ethics as laid down by the Medical Council of India. The filled questionnaire were obtained from the participants. The first part of the questionnaire contained the information sheet for the participants regarding medical ethics and the present study and also how important it was to create an awareness among the medical practitioners. The second part of the questionnaire covered the demographic details and the consent form which was mandatory to be filled by the participants. The third part of the questionnaire contained questions about their awareness, their knowledge of the existence of the ethics committee and its role; their attitudes on various issues such as consent and confidentiality, privacy, euthanasia, intimate examination and their knowledge of the code of medical ethics of the Medical Council of India. After obtaining the data, it was entered in excel spreadsheet and was analysed using SPSS software and frequencies and percentages were obtained.
Result

Among 100 participants whose responses were analysed, 37% were male and 73% were female.

The graph (figure 1) given below describes the students’ attitude to medical ethics. 94% were positive about the importance of ethics. Out of them, 13% thought that ethics was “extremely important”. However, four medical practitioners, thought that such knowledge was “not important”.

![Graph: Student’s attitude towards medical ethics]

Figure 1 – Student’s attitude towards medical ethics

Seminar and workshop (40%) and clinical training (36%) were considered the predominant sources of knowledge on ethics. 30% from journals, 21% from ethical books, 15% from lectures, 15% from newspaper and 15% from television gained their knowledge about ethics.

The graph (figure 2) given below describes that only 28% agreed and 6% were uncertain as to whether “Ethical conduct is important only to avoid legal action”.

![Graph: Importance of ethical conduct]

FIGURE 2 – Importance of ethical conduct

The graph (figure 3) given below describes that 71% of the participated medical practitioners agreed that the patient’s wishes must always be adhered to during the course of treatment. On the other hand, 44% of the participants had a opinion that “The doctor should do what is best irrespective of the patient’s opinion”. 89% agreed that children should never be treated without the consent of their parents or guardian except in case of emergency. 90% of the participants agreed that the doctor should get an informed written consent from the patient, if he/she refuses to take the treatment offered by the doctor. 83% of the participated medical practitioners disagreed that a doctor should not attend seriously injured and accidental cases before the arrival of the police or registering the cases and completing other formalities.

![Graph: Importance of consent]

FIGURE 3 – Importance of consent

56% of the participants agreed that the patient has a right to refuse life saving medical treatment (passive voluntary euthanasia) while 37% disagreed with it. 55% agreed that they will refer the patient to another doctor for religious conflict situations. 30% agreed that for the benefit of the larger group, privacy of one patient can be ignored while 66% disagreed and 4% were uncertain.

The chart (figure 4) given below describes that 25% of the participants opined that the information about the patient can be revealed to more than one person”, while the other 65% of the participants disagreed and the other 10% were uncertain.

![Graph: Information about the patient]

FIGURE 4 – Information about the patient
The table 1 given below describes the medical practitioner’s knowledge of the code of conduct of the Medical Council of India, to be followed once they graduate. The chart shows that 58% disagreed that it was appropriate for doctors to print their photograph on their signboard, while 32% agreed and 10% were uncertain about it. 72% disagreed that doctors can run an open shop for dispensing drugs and appliances prescribed by other doctors. 84% of the participants thought that it was necessary for a doctor to maintain medical records of his/her patients for 3 years from the date of commencement of treatment.

### Table – 1: Knowledge of the code of conduct of the MCI

<table>
<thead>
<tr>
<th>Question</th>
<th>Agree</th>
<th>Disagree</th>
<th>Uncertain</th>
</tr>
</thead>
<tbody>
<tr>
<td>A physician may print his or her photograph in the sign board, along with qualification and specialty</td>
<td>32%</td>
<td>58%</td>
<td>10%</td>
</tr>
<tr>
<td>A physician may run an open shop for dispensing drugs and appliances prescribed by other doctors</td>
<td>20%</td>
<td>72%</td>
<td>8%</td>
</tr>
<tr>
<td>A medical practitioner can make a formal announcement in press regarding starting of practice, changing address, change of type of practice, temporary absence from duties and resumption of practice</td>
<td>40%</td>
<td>44%</td>
<td>16%</td>
</tr>
<tr>
<td>A physician should maintain medical records of his or her patients for 3 years from date of commencement of treatment</td>
<td>84%</td>
<td>14%</td>
<td>2%</td>
</tr>
</tbody>
</table>

### Discussion

Ethical conflicts are common during the initial years of a medical professional’s career. Thus, teaching and training about medical ethics is very important among the medical professionals. The findings of the present study shows the knowledge and awareness about medical ethics among the medical practitioners in Saveetha Medical College, Chennai. The participants of this study included both doctors working in clinical and non-clinical departments of the college.

Most of the participants had knowledge and awareness about medical ethics. The responses are reflective of categories such as different subspecialties, i.e. medical, surgical, postgraduates and gender. The respondents had obtained their knowledge of ethics from various sources. It appeared that their clinical training and seminars & workshops was the key source of knowledge.
The questionnaire contained questions about their awareness of ethics and its importance, and the source of this awareness; their attitudes on various issues such as consent and confidentiality, privacy, euthanasia, intimate examination and their knowledge of the code of medical ethics of the Medical Council of India.

The knowledge of ethics and law was obtained from same source by most of the respondents. It is also interesting to note that the source of knowledge of medical ethics amongst the physicians during training and seminars & workshops appeared to be important than the experience at work and one’s own reading. While in the previous study, the source of knowledge from clinical training was less important than the experience at work, lectures and seminars and one’s own reading. Around 70% of the respondents knew about the code of conduct of the Medical Council of India.

The fact that many physicians did not feel that the patient’s wishes should be adhered to at all times, shows the lack of knowledge of the basic principles of medical ethics. On the other hand, many of the participants felt that “A doctor should do what is best irrespective of the patient’s opinion”. The lack of knowledge of basic principles of medical ethics in the present study is similar to another study regarding the healthcare ethics and law among doctors and nurses in Barbados. Around 1/3rd of the participants felt that the patient does not have a right to refuse life-saving medical treatment (Passive voluntary euthanasia) in India, which is contraindicated with the law.

Most of the physicians who took part in the present study, took informed consent and maintained confidentiality during clinical teaching. In another study, a lack of taking informed consent from the patient was seen. On the other hand, several reports suggest that surgical residents often do not proceed with an optimal consent process because of time constraints. These studies highlight the need to better educate surgical residents in the operative procedures in a way that enables them to obtain meaningful consent. Breach of confidence by a doctor is a highly unethical practice, especially on issues of confidentiality vs. law, communicable disease confidentiality vs. spouse knowledge of the disease. The majority of the ethics committees focus on the ethical protection of human beings in research settings. The existence of clinical ethics committees in hospitals is very important for moral deliberation on clinical cases. 

We could assess the basic knowledge and attitudes of medical practitioners regarding medical ethics in order to obtain basic information for better training and improvising the knowledge about ethics in the medical curriculum. This study has the limitation that it does not cover a wider range of medical practitioners.

**Conclusion**

Overall, this study reflects the current situation of knowledge, attitudes and practice of ethics by clinicians in Saveetha Medical College, Chennai, where ethics is taught as a part of a subject at the undergraduate level. Many steps have been taken to bring more awareness among the physicians by introducing Hospital Ethics Committees in many institutes, ethics awareness programmes etc., This study highlights the lack of improvement in ethics awareness corresponding to increasing exposure to medical education in the last few years. It is important to gather basic information on ethics awareness to know if the medical practitioners have better knowledge. To strengthen ethical reasoning and judgment in decision making, clinically oriented measures like case studies, seminars, interactive workshops, is needed. Thus, these can help us to bring more awareness about medical ethics among the physicians.

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**Ethical Clearance:** Obtained from institutional ethical clearance board.

**References**

Effectiveness of Bosu Ball Exercises Versus Thera Band Exercises on Core Stabilization and Balance Performance

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Abstract

Background: Greater core stability provides a foundation for greater force production in the upper and lower extremities. When the core muscles are functioning optimally, the person will be able to safely perform specific athletic or other functional activities. Core muscle imbalances are the cause of core instability and reduced balance performance. Few studies have shown the effect of swiss ball exercises on core stabilization and balance performance as well as few studies also shown the comparison between core exercise on stable and unstable surfaces for core stabilization and balance performance. The core stability and balance performance needs to be treated by strengthening core muscles.

Objectives: To find the effect of 2 different types of strengthening exercises on core stability and dynamic balance in collegiate athletes.

Material and Method: In this pre-post intervention study 26 athletes were included. They were randomly divided into 2 groups with 13 individuals in each group. Group A was instructed to perform BOSU ball exercises and group B was instructed to perform thera band exercises for 6 weeks. After pre-post assessment, data was analysed with the help of appropriate statistical methods.

Results: According to the results there is significant increase in both right and left anterior, posteromedial and posterolateral directions with p value <0.0001 as well as in core stability with p value <0.0001

Conclusion: Both BOSU Ball exercises and thera band exercises are found to be equally effective in improving core stability and balance performance in collegiate athletes.

Keywords: BOSU ball exercises, thera band exercises, Y balance test, flexor and extensor endurance test, side bridge test, single leg stance test.

Abbreviations: BOSU ball: Both Sides Up ball

Introduction

The core is a muscular corset, which includes deep and muscles of the trunk1,2. It consists of abdominal muscles in the front, gluteals and paraspinal muscles in the back, the diaphragm at the top, and the pelvic floor and hip muscles at the base3. The core is also referred as the lumbo-pelvic region4. They used to stabilize the spinal column, enhance performance when the extremities move and for align the body1. Core stability is defined as the level of strength or endurance in particular muscle groups of lumbo-pelvic complex5. Increased stability in core is produced by contraction of superficial and deep muscles, made up of both slow and fast twitch muscles. The local (deeper) muscle group are

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transverse abdominis, multifidus, and pelvic floor. They composed of type 1 (slow twitch) muscle fibers. They help to improve trunk endurance. The global (superficial) muscle group are rectus abdominis, obliques, latissimus dorsi, and erector spine. They composed of type 2 (fast twitch) muscle fibers. They control flexion and extension of trunk. The core muscle weakness causes reduced proprioception, reduced balance, reduced strength, muscle imbalance, reduced endurance, reduced flexibility, etc.

Core strengthening is the provision of muscular control around the lumbar spine to maintain functional stability. Strengthening of core muscles has strong central effect in the body, as well as in increasing stability of the trunk to extend during reach and functional activities. Core exercises are the exercises to train the muscles that stabilize and control the movements of abdomen, waist, and hip. There are different exercises to strengthen core muscles includes the crunch exercise, the bridge exercise, and the plank exercise. There are many treatment options for core strengthening such as pilates, tai chi, yoga, graded abdominal exercises, and resistance training. Use of thera band improves flexibility, mobility, daily activities, metabolic rate, and for improving athletic performance. Elastic resistance band is a unique type of resistance training. It is used in sports and rehabilitation medicine for improving muscular strength and endurance. The resistance of elastic band depends upon the amount of band is stretched. Elastic bands are available in different colours according to assortment of grades. This colour coding denotes the thickness of product and grade of resistance. These colour coding denotes the resistance levels. It includes following colours: yellow (thin), red (medium), green (heavy), blue (extra heavy), silver (super heavy), golden (max). Use of thera band improves flexibility, mobility, daily activities, metabolic parameters, etc. The exercises performed with thera band are curl up, bridging, side bridging, quadruped stabilization, push ups, etc.

Core stability is important for injury prevention and for improving athletic performance. There are
different tests to check core stability. It includes trunk stability test, plank test, sahrmann core stability test, unilateral hip bridge endurance test, flexor endurance test, extensor endurance test, side bridge test, etc\textsuperscript{14}. The reliability of flexor endurance test (0.93), extensor endurance test (0.97), side bridge test (0.91) is more as compared to others\textsuperscript{17,19,20}.

Balance ability has correlation with athletic performance and sports injury risk\textsuperscript{16}. Static balance can be assessed by single leg stance test, tandem stance test, functional reach test, flamingo test, but the reliability of single leg stance test (0.61-0.81) is more as compared to other tests\textsuperscript{15}. Dynamic balance can be assessed by timed up and go test, star excursion balance test, y balance test, dynamic leap and balance test, modified bass test, single leg squat test, single leg hop test, but the reliability of Y balance test (0.80-0.85) is more as compared to others\textsuperscript{15,20}.

**Methodology**

The ethical clearance was taken from ethical committee of Krishna institute of medical sciences, Karad. There were 26 participants in the study. The study was taken place in the Physiotherapy department, Krishna institute of medical sciences. The study was effectiveness of BOSU ball exercises versus thera band exercises on core stability and balance performance. The treatment protocol was of 6 weeks. The subjects were assessed for core stability and balance at first. The type of study was experimental study. The study design was pre and post. The samples were chosen using the simple random sampling method. Participants were included as per inclusion criteria.

**Procedure**

All the subjects were selected for the study according to the selection criteria. Demographic data and consent from was taken from them. Included participants divided in 2 groups by simple random sampling method. Pre and post assessment were taken after 6 weeks of the treatment respectively with the help of outcome measures.

Group A received BOSU ball exercises. Participants were instructed to perform Seated crunches, Plank, Bridging, Hip extension and knee flexion prone ball hold with knee drive. This exercises were performed for 6 weeks, 3 sets of 15 repetitions in the 1\textsuperscript{st} week; 4 sets of 15 repetitions in the 2\textsuperscript{nd} week; 4 sets of 20 repetitions in the 3\textsuperscript{rd} and 4\textsuperscript{th} weeks; and 4 sets of 25 repetitions in the 5\textsuperscript{th} and 6\textsuperscript{th} weeks.

Group B received thera band exercises. Participants were instructed to perform Curl up, Bridging, Side bridging, Push ups, Lower abdominal crunches.

Session will consist of: 3 sets of 15 repetitions in the 1\textsuperscript{st} week; 4 sets of 15 repetitions in the 2\textsuperscript{nd} week; 4 sets of 20 repetitions in the 3\textsuperscript{rd} and 4\textsuperscript{th} weeks; and 4 sets of 25 repetitions in the 5\textsuperscript{th} and 6\textsuperscript{th} weeks

The effect of the treatment given to each group was noted immediately using the outcome measures. The experimental results were statistically analysed. The significant difference between the two groups was investigated with the un-paired t test and within the group with paired t test.

**Results**

Pre and post data was analysed, according to the result the within the Group A the flexor endurance test, extensor endurance test, side bridge test, single leg stance test, and Y balance test are considered extremely significant with p value <0.0001.

1. **Flexor endurance test:**

<table>
<thead>
<tr>
<th>Table no.1: pre and post results of flexor endurance test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group A</strong></td>
</tr>
<tr>
<td>---------------</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
</tr>
<tr>
<td><strong>SD</strong></td>
</tr>
<tr>
<td><strong>P value</strong></td>
</tr>
<tr>
<td><strong>t value</strong></td>
</tr>
</tbody>
</table>
2. Extensor endurance test:

**Table no.2: pre and post results of extensor endurance test**

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th></th>
<th>Group B</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Mean</td>
<td>2.69</td>
<td>6.38</td>
<td>3.23</td>
<td>6.69</td>
</tr>
<tr>
<td>SD</td>
<td>1.60</td>
<td>3.07</td>
<td>1.87</td>
<td>2.65</td>
</tr>
<tr>
<td><em>P</em> value</td>
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<td>&lt;0.0001 (extremely significant)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>t</em> value</td>
<td>6.89</td>
<td></td>
<td>11.07</td>
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</tbody>
</table>

3. Side bridge test:

**Table no.3: pre and post results of side bridge test**

<table>
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<th>Group A</th>
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<th>Group B</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Left</td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td>Mean</td>
<td>21.07</td>
<td>23</td>
<td>30.15</td>
<td>32.84</td>
</tr>
<tr>
<td>SD</td>
<td>8.91</td>
<td>7.17</td>
<td>9.24</td>
<td>7.75</td>
</tr>
<tr>
<td><em>P</em> value</td>
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<td>&lt;0.0001 (extremely significant)</td>
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</tr>
<tr>
<td><em>t</em> value</td>
<td>L=7.95, R=10.63</td>
<td></td>
<td>L=17.20, R=11.10</td>
<td></td>
</tr>
</tbody>
</table>

L= left, R= right

4. Single leg stance test:

**Table no.4: pre and post results of single leg stance test**

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th></th>
<th>Group B</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>EO</td>
<td>EO</td>
<td>EC</td>
<td>EO</td>
<td>EC</td>
</tr>
<tr>
<td>Mean</td>
<td>7.84</td>
<td>5.15</td>
<td>11.53</td>
<td>7.92</td>
</tr>
<tr>
<td>SD</td>
<td>2.19</td>
<td>2.11</td>
<td>2.75</td>
<td>2.39</td>
</tr>
<tr>
<td><em>P</em> value</td>
<td>&lt;0.0001 (extremely significant)</td>
<td>&lt;0.0001 (extremely significant)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>t</em> value</td>
<td>EO=10.64, EC=12</td>
<td></td>
<td>EO=16.43, EC=5.61</td>
<td></td>
</tr>
</tbody>
</table>

EO= eyes open, EC= eyes closed
5. **Y balance test:**

Table no.5: pre and post results of Y balance test for right leg

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th></th>
<th>Group B</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td></td>
<td>Ant</td>
<td>PM</td>
<td>PL</td>
<td>Ant</td>
</tr>
<tr>
<td>Mean</td>
<td>57.17</td>
<td>57.56</td>
<td>57.20</td>
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<tr>
<td>SD</td>
<td>7.38</td>
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<td>6.46</td>
<td>7.41</td>
</tr>
<tr>
<td>P value</td>
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<td></td>
<td>&lt;0.0001 (extremely significant)</td>
<td></td>
</tr>
<tr>
<td>t value</td>
<td>Ant=11.48, PM=9.92, PL=9.54</td>
<td></td>
<td>Ant=8.44, PM=7.86, PL=6.61</td>
<td></td>
</tr>
</tbody>
</table>

Ant= anterior, PM= posteromedial, PL= posterolateral

Table no.6: pre and post results of Y balance test for left leg

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th></th>
<th>Group B</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td></td>
<td>Ant</td>
<td>PM</td>
<td>PL</td>
<td>Ant</td>
</tr>
<tr>
<td>Mean</td>
<td>55.53</td>
<td>55.68</td>
<td>55.36</td>
<td>57.79</td>
</tr>
<tr>
<td>SD</td>
<td>6.72</td>
<td>6.53</td>
<td>6.61</td>
<td>7.19</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.0001 (extremely significant)</td>
<td></td>
<td>&lt;0.0001 (extremely significant)</td>
<td></td>
</tr>
<tr>
<td>t value</td>
<td>Ant=11.97, PM=10.06, PL=12.34</td>
<td></td>
<td>Ant=9, PM=7.06, PL=6.74</td>
<td></td>
</tr>
</tbody>
</table>

Ant= anterior, PM= posteromedial, PL= posterolateral

**Discussion**

The aim of this study was to study the effect of BOSU ball exercises versus theraband exercises on core stabilization and balance performance in athletes with an objective of finding the benefits of each exercise and comparing its effects to fulfil the aim of the study.

The individuals included in this study were collegiate athletes which were assessed and scored according to the outcome measure were included in the study.
The study was taken place in Physiotherapy department, Krishna institute of medical sciences. There were 26 participants selected according to the criteria and they were randomly divided into 2 groups (group A and group B) with each containing 13 participants. Group A was asked to perform BOSU ball exercises according to given procedure and group B was asked to perform thera band exercises. This study was carried out for 6 weeks and 3 times per week and pre and post assessment was taken using outcome measures after 6 weeks. After the pre and post assessment the data was statistically analysed.

According to this both the exercise groups appeared to be equally effective in improving the core stabilization and balance performance in the participants and were considered extremely significant with p value <0.0001.

According to a literature variety of exercises must have been used in improving balance and core stability. Some literature suggests that the swiss ball exercises were helpful in improving the core stability and balance performance\(^3,4\). Some studies also proved that BOSU ball trainer is useful for ankle muscle activation and for improving balance\(^10\).

**Acknowledgement:** I sincerely thank the management of KIMSDU for allowing me to conduct this study by providing me the necessary requirements. I thank dean Dr Varadharajulu sir for his support and guidance. I convey sincere thanks to my guides Dr Khushboo Chotai ,Dr Smita Patil and Dr Amrutkuvar Rayjade for helping me in my research. I take this opportunity to thank all those who have been directly or indirectly involved for smooth conduction of my study.

**Conclusion**

On the basis of the result of the study it is concluded that the BOSU ball exercises and thera band exercises appears to be equally effective for core stabilization and balance performance in athletes.

**Source of Funding**

Source Of Funding: Krishna Institute Of Medical Sciences Deemed to be University, Karad.

**Conflict of Interest:** There were no conflicts of interest in my study.

**Ethical Clearance:** The Institutional Ethics committee has hereby given permission to initiate the research project titled, “Effect of BOSU ball exercises versus thera band exercises on core stabilization and balance performance”

**References**


A Novel Method of Establishing the Identity of an Individual by Analyzing the Pattern & Volume of the Frontal Sinus Using Computerized Tomogram – A Retrospective Cross Sectional Study

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¹PG Student, ²Prof & Head, Department of Oral Medicine & Radiology, Sree Balaji Dental College & Hospital, Chennai

Abstract

Background: The paranasal sinuses develop early in fetal life. Among the 4 major sinuses present in human skull, Frontal sinus helps in identification of an individual because of its uniqueness and so has the potential to be used as an aid for personal identification in disasters, accidents and natural calamities.

Aim and Objective: To determine the volume & shape of frontal sinus in individuals using CT scan and to establish the uniqueness of frontal sinus in the identification of individuals in forensic science.

Methodology: CT images showing the axial and coronal sections of frontal sinus of patients above the age of 20 years were considered as inclusion criteria and patients below the age of 20 years, previous injury, surgery or pathology in the frontal bone and frontal sinus were excluded. The length, breadth and height of right & left frontal sinus were measured and volume of the frontal sinus was calculated. The pattern of the frontal sinus was analysed in the axial & coronal sections of CT scan by taking screen shots of the slice. The pattern of the frontal sinus was drawn and copied to Microsoft Power Point Software for superimposing over other patterns.

Results: Among the 270 scans collected, the total length, breadth, height and volume of left and right side of male and females were compared by using Independent samples t-test and it showed that the frontal sinus was unique in nature.

Conclusion: The observations showed that no two individuals had the same sinus morphology based on division, volume, position and symmetry of frontal sinus.

Key words: CT scan, Frontal sinus, Volume, Pattern, Unique.

Introduction

The term “Forensic” is derived from Latin word ‘forum’ where legal matters are discussed. Odontology refers to the study of teeth or dentistry¹. Federation Dentaire Internationale[ FDI] defined forensic dentistry as ‘The branch of dentistry in the interest of justice that deals with proper handling and examination of dental evidence with the proper evaluation and presentation of dental findings’ ².

Forensic odontology plays a major role in the identification of an individual by using traditional methods such as bite marks, rugae pattern, dental profiling, assessment of DNA. When these traditional methods are unfeasible, the para nasal sinuses that develop early in fetal life might serve as a reliable guide for identification.
Among the four major sinuses, frontal sinus plays a greater role in the identification of an individual. These sinuses are pneumatic cavities that become radiologically evident at the age of five or six years and develop fully by the age of 20 years. Like fingerprints, frontal sinus patterns are unique for a person. Several authors have reported the uniqueness and its importance in human identification.

Though there are many ways in visualizing the frontal sinus, a single CT image gives a three-dimensional view of the sinus. The aim of the present study is to determine the volume & shape of frontal sinus in individuals using CT scan and to establish the uniqueness of frontal sinus in the identification of individuals in forensic science.

**Material and Method**

After obtaining the ethical clearance from the Institutional Ethical Committee, a retrospective study was performed using 270 Head and Neck CT images of patients that were previously taken for some therapeutic purpose. CT images of patients of age above 20 years were used and CT images of patients below the age of 20 years and the images with evidence of previous injury, surgery or pathology in the frontal bone and frontal sinus were excluded. Informed consent was also obtained.

The slice containing the axial and coronal sections of CT’s of individuals were selected in such a way that complete radiolucency is seen in the region of frontal sinus and the pattern and volume were analysed.

The following measurements were taken in the axial & coronal sections for measuring the volume of frontal sinus:

- Height of right & left frontal sinus
- Length & breadth of right & left frontal sinus
- Volume of the frontal sinus

For analysing the pattern of frontal sinus the outline of the pattern was drawn by taking screen shots (Fig 1, 2). The pattern of sinus was compared to one another by using Microsoft PowerPoint software by:

1. Copping, pasting the cropped image on a PowerPoint presentation slide.
2. The background of the image removed by formatting the image. (This will make the image radiopaque in the sinus region.)

The pattern was compared by superimposing each image.

**Results**

A total of two hundred and seventy PNS Head and Neck CT images (axial and coronal slices) were selected following the selection criteria out of which 155 (57.41%) were males and 115 (45.59%) were females. Out of the 270 images examined the frontal sinuses were present in 266 (98.52%) people and absent bilaterally among 4 (1.48%). Among the 266 subjects, the frontal sinus was unilateral in 14 (5.27%) and bilateral in 252 (94.7%) (Tab 1). The unilateral frontal sinus was more common on the right side (4.81%) than the left side (0.37%). (Tab 1). Division of the sinus was seen in 246 (92.48%) and absence of division was observed in 20 (7.52%) (Tab 1). The symmetry of the sinuses were compared in 232 subjects who had bilateral presence of frontal sinus, by analysing the pattern and simultaneously the volume and was found to be symmetrical in 18 and asymmetrical in 214. While comparing the pattern of the sinuses it was found to be unique in 98.89% (i.e. among all the subjects with presence of sinus). The commonest shape of the front sinus seen in the coronal section was club shaped.

The total length, breadth, height and volume of left and right side of male and females were compared. Independent samples t-test was performed by calculating the mean, SD and SEM. While comparing the volume of right and left side of sinus, volume of the right frontal sinus was more in males whereas it was vice versa in females. The mean total volume of males was greater than that of females, which implies that the sinuses are larger in size in males (Tab 2).

The uniqueness of frontal sinus was analysed by comparing the pattern of the sinus among 266 subjects by superimposing the images in Microsoft paint software.

The following observations showed that no two individuals had the same sinus morphology.

1. The patterns were unique between each individual.
2. Divisions within the sinus varied.
3. Measurements such as length, breadth, height or volume were different between each individual.
4. Positions of the sinus (unilateral/ bilateral) varied.
5. Symmetry of the sinus varied.
Table 1: Statistics showing distribution of the presence/absence, pattern, division between the sides.

Table 2: Statistics showing Length, Breadth and Height of right and left side of frontal sinus in male and female

Table 1: Statistics showing distribution of the presence/absence, pattern, division between the sides.

<table>
<thead>
<tr>
<th>Position</th>
<th>Unilateral Right</th>
<th>Unilateral Left</th>
<th>Bilateral</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>Row %</td>
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<tr>
<td>Pattern</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence</td>
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<td>100.00</td>
<td>4.89</td>
</tr>
<tr>
<td>Absence</td>
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<td>.00</td>
<td>.00</td>
</tr>
<tr>
<td>Sinus/No Sinus</td>
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<td></td>
<td></td>
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<tr>
<td>Sinus</td>
<td>13</td>
<td>100.00</td>
<td>4.89</td>
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<td>.00</td>
<td>.00</td>
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<tr>
<td>Division</td>
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<tr>
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<td>.00</td>
<td>.00</td>
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Table 2: Statistics showing Length, Breadth and Height of right and left side of frontal sinus in male and female

<table>
<thead>
<tr>
<th>Gender</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
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<td></td>
</tr>
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<td>21.24</td>
<td>9.03</td>
</tr>
<tr>
<td>Length – Left</td>
<td></td>
</tr>
<tr>
<td>22.00</td>
<td>8.34</td>
</tr>
<tr>
<td>Breadth – Right</td>
<td></td>
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<td>24.37</td>
<td>8.68</td>
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<tr>
<td>Breadth – Left</td>
<td></td>
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<td>23.90</td>
<td>7.90</td>
</tr>
<tr>
<td>Height – Right</td>
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</tr>
<tr>
<td>12.06</td>
<td>6.10</td>
</tr>
<tr>
<td>Height – Left</td>
<td></td>
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<tr>
<td>11.96</td>
<td>4.22</td>
</tr>
<tr>
<td>Volume – Right</td>
<td></td>
</tr>
<tr>
<td>7726.66</td>
<td>9630.82</td>
</tr>
<tr>
<td>Volume – Left</td>
<td></td>
</tr>
<tr>
<td>6877.21</td>
<td>6318.19</td>
</tr>
<tr>
<td>Volume – Total</td>
<td></td>
</tr>
<tr>
<td>14834.84</td>
<td>12449.13</td>
</tr>
</tbody>
</table>
Figure 1: Showing the cropped pattern of frontal sinus in Axial section

Figure 2: Showing the cropped pattern of frontal sinus in Coronal section.

Figure 3: Bar chart showing the uniqueness of frontal sinus.

Discussion

Every individual is unique. The main aim of forensic science is identification of unknown human being in crime and disasters by using numerous technologies. Forensic odontology is a branch of forensic science which has established itself as an important and often indispensable science in medicolegal matters and identification of the dead. Methods like rugoscopy, bite marks, palatal rugae, photographs, lip prints, etc. are used for identifying the individuals.

Finger prints are more useful in identifying a person as they are unique. But there are situations where the other unique features of an individual must be used in identification. The one among the most interesting part
in the head and neck region of a human is Paranasal sinuses. The frontal sinus does not change in its shape after the age of 20 years\(^6\) except during trauma or due to pathology.

The aim of the present study was to prove that frontal sinus is unique to each individual based on their pattern and volume, three dimensionally. Similar studies have been done previously using two dimensional radiographs where the length and breadth of the frontal sinus were measured. Schuller\(^7\), Culbert and Law\(^8\) were the first to propose the possibility of utilizing radiological images of frontal sinus in identification of an individual by studying its morphology, followed by Asherson\(^9\) and Gerald Quatrehomme\(^10\) who examined monozygotic and dizygotic twins and found that the pattern of frontal sinus was different in each of them.

In this study a total of 270 CT scans were analysed and it showed that 98.5\% participants had frontal sinus which was in accordance with Ertugrul Tatlisumak\(^11\) and the bilateral absence of sinus was seen in 1.4\% i.e. bilateral absence of frontal sinus.

The pattern of the sinus was compared with one another by superimposing the outline of the sinus, on Microsoft paint software. None of them matched each other which showed that the pattern of frontal sinus was unique. This was in accordance to other studies done by Schller\(^7\), Culbert\(^8\), Asherson\(^9\), Gerald Quatrehomme\(^10\), Neha Patil\(^12\) where they have compared the shape of frontal sinus and proved that frontal sinus is unique for each individual in a two dimensional radiographic view. Chetan Belaldavar\(^13\) studied the frontal sinus in a three dimensional way and concluded saying that it has a unique pattern for each individual.

In this study bilateral presence of sinus was seen in 94.74\%, unilateral presence on right side was seen in 4.89\%, unilateral presence on left side in 0.38\%, division of sinus was seen in 92.48\% and no division in 7.52\% which was in accordance to the study by Neha Patil\(^12\) who showed 1\% bilateral aplasia and 3\% unilateral aplasia of frontal sinus. A study by Nateghian Z\(^14\) showed that right and left frontal sinus was found in 67.7\% of the individuals. Right frontal sinus was observed in 4.6\% and left frontal sinus was seen in 9.2\% of the individuals. Studies by Saraswathi Gopal\(^15\), Vidya CS\(^16\) showed bilateral absence of frontal sinus is 5\%, unilateral aplasia in 2.5\%. In Roberto Camerier\(^1\) study out of 99 individuals he found that, there was bilateral aplasia in 10\%, unilateral aplasia in 2\%. A study by MP David\(^18\) showed that there was 6\% unilateral and 4\% bilateral aplasia. The division of the sinus was not compared in other studies.

On comparing the bilateral symmetry of frontal sinus in our study, symmetry was seen in 7.76\% and asymmetry in 92.2\% which was in dispute to Vidya CS\(^16\) who observed that 68\% had symmetry and asymmetry was seen in 30\% in her study.

This study showed that sinus was larger in males than females which were in accordance with Chetan Belaldavar\(^13\) and Hemant Mathur\(^19\). In our study the volume of left and right sinus was compared and it was found that volume of the right sinus was greater in males and vice versa in females.

In the present study out of 270 scans viewed, only 4 showed bilateral aplasia which was present in males. The unilateral presence of frontal sinus was present in 5.18\%, out of which it was more common in right side for males like in other studies. The symmetry of sinus in each individual was compared, where 232 frontal sinuses out of 270 sinuses were compared because bilateral aplasia of sinus was seen in 4 subjects and 14 subjects had unilateral sinus (where the symmetry cannot be assessed). In the present study it is shown that only 7.76\% of subjects had symmetrical sinus. But a study done by MP David\(^18\) and Saxen\(^20\) showed symmetry in 58\% of frontal sinus.

From the present study it is clear that frontal sinus is unique for each individual (Fig 3) and using the antimortem and post-mortem records of frontal sinus, individuals can be identified. Further gender can also be determined, as the frontal sinuses are larger in males.

Limitations of this study : The frontal sinus cannot be used in identification of an individual when they are completely absent or due to any pathologies affecting the sinus. The frontal sinuses are not present during the early stages of life and cannot be seen radiographically till the age of seven years.

**Conclusion**

To identify is to determine the individuality, or it is to prove by means of technical or scientific means that a person is that one & not the another. Human identification is not a difficult task, when it is about a live individual or a cadaver chronologically recent and
After the advent of Computerized Tomographic scan, it is possible to measure the linear and volumetric measurements accurately. In this study an attempt was made to establish the uniqueness of frontal sinus in the identification of an individual using CT scan and it was found that frontal sinus was unique in each individual and it can be used as fingerprint when they are not altered.

**Source of Funding** – Self

**Conflict of Interest** - Nil

**References**


18. MP David, Saxena R. Use of Frontal sinus and nasal septum as an aid in personal identification:

Study of Adverse Drug Reactions (ADRs) Occurring with the Drug Use in a Tertiary Hospital

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Abstract

Aim: To study the adverse drug reactions (ADRs) occurring with the drug use in a tertiary care hospital.

Materials and Method: This observational study conducted over a period of four months (01-Nov-2015 to 29—Feb-2016). The Central Drug Standard Control Organization (CDSCO) suspect ADR forms (15) were distributed to all clinical departments personally in Kerala medical college hospital and research center, Mangode, Cherpullaserry. Regular visits were carried out twice a week for collecting data reports. They were then analyzed, compared with state data, national data, and international data. Results A total of 25 suspected Adverse Drug Reactions forms were reported during the period of four months of the study of the out patients and in patients departments of the hospital. Among all drugs NSAIDS, followed by Antimicrobials, Antipsychotics, Bronchodilators, Antihypertensive and oral hypoglycemic agent etc. were reported to have adverse drug reactions. Most common route of adverse drug reactions was oral, followed by IV, IM, S/C, Topical and Inhalational routes. Reactions mostly seen affecting Skin, Gastrointestinal systems, Central nervous system, and Hematological system Most patients recovered from adverse events taking suitable measures like complete stopping the offending agent, or were prescribed antihistamines, steroids in addition Conclusion: Awareness about ADR reporting is still poor amongst healthcare professionals in India. The incidence and severity of Adverse Drug Reactions documented in our study are lower than those reported in other studies. NSAIDS comprise the major drug family associated with adverse drug reactions so should be rationally prescribed. Improved communication between the physicians and nurses with the pharmacovigilance centre in the hospital is suggested.

Key words: Adverse drug reactions, CDSCO, Pharmacovigillance

Introduction

According to WHO definition an Adverse Drug Reaction (ADR) is a response to a drug that is noxious and unintended, and occurs at doses normally used in human for the prophylaxis, diagnosis, and treatment of disease or modification of physiological function.1

Ultimately pharmacovigilance is concerned with identifying the hazards associated with pharmaceutical products and with minimizing the risk of any harm that may come to the patient.

The safety of prescription drugs represents a major public health concern. Adverse drug reactions are considered to be one of the leading causes of death among hospitalized patients.2 Previous studies suggest that approximately 0.5% of all emergency department visits and tertiary care visits result from adverse drug reactions.

However, low- to middle income countries, which represents more than two thirds of the world population account for a tiny fraction of all the adverse drug reaction data. Among the reasons for this under-reporting are the difficulty that the patients and the providers may have...
liking a particular symptom or condition to a specific drug particularly for individuals who have chronic illness and are taking several medications, a common clinical scenario in tertiary care. Thus, there is a need to capture safety data for drugs in a country like India.

**Documentation of Adverse Drug Reactions:**

The significant adverse reaction of any drug should be notified within seven days. The other facts related to adverse events should be informed within eight days. The drug reaction form can be collected at pharmacovigilance center.

The filled adverse drug reaction form can be submitted to the peripheral pharmacovigilance center. After reviewing the form, the center forwards it to the regional center and after that it is propelled to zonal center.5,6,7

This program is overseen by the central drugs standard control organization. (CDSCO).8,9

Patient reporting has been incorporated into pharmacovigilance systems in several countries including U.S.A, Canada, Australia, Newzeland Denmark, Sweden, and the Netherlands. Until very recently, however, patients in the U.K. were not able to report directly suspected Adverse Drug Reactions to Medicine and Health Care Regulatory Agency (MHRA), although some organizations has been proposing this for several years. In 2001, the UK Consumer Association called for patient reporting to be introduced after highlighting the fact that doctors were often failing to pass on information about suspected adverse reactions to drugs to MHRA. 10

**Aim of the study**

To study the Adverse Drug Reactions occurring with the drug use in tertiary care hospital

**Objective of the study**

1. Critically evaluate the Adverse Drug Reactions occurring with the use of drugs in a tertiary care hospital over a period of 4 months (2015-16)

2. To establish the causality of Adverse Drug Reactions occurring with the use of drugs in the tertiary care hospital using the WHO causality assessment scale and the Naranjo causality assessment scale.

3. To compare the incidences of Adverse Drug Reactions occurring in tertiary hospital, with state data, and international (global) data.

**Methodology**

This observational study is to be conducted over a period of 4 months (01-Nov-2015 to 29—Feb-2016). Permission obtained from the Review and approval by the Institutional Ethics Committee, to conduct the study. An introductory lecture is organized in the academic society of the institute to orient the clinicians towards pharmacovigilance and spontaneous reporting system. The Central Drug Standard Control Organization (CDSCO) suspect Adverse Drug Reactions forms (downloaded from CDSCO website) was distributed to all the clinical departments personally in Kerala medical college hospital and research center, Mangode, Cherpullassery. The forms contains the patient details, The description of the reactions, concomitant medication, coexisting illness, any rechallenge, dechallange etc.

On receiving information from the clinical departments, visit to the hospital and interact with the doctors to gather complete information on the Adverse Drug Reactions. The suspected Adverse Drug Reactions will be carefully analyzed and documented. Apart from this, regular visits will be carried out in respective departments and forms were collected twice in a week for analyzing the data and comparing the incidences of Adverse Drug Reactions occurring in a tertiary care hospital, with State data, national data and international (global) data

**Results**

Out of 25 cases reported with Adverse Drug Reactions in 4 months period, NSAIDS was found to be the most common implicating agent followed by Antimicrobials, Antipsychotics, Antacids, Vitamins and Minerals, Bronchodilators, Antihypertensive and oral hypoglycemic agent etc. were reported to have adverse drug reactions.

Most Affected organ system was Skin, followed by Gastrointestinal systems, Central nervous system, and Hematological system.

According to Naranjo’s Causality assessment scale(16) , 18 (72%) cases were Definite, 4 (16%) Probable and Possibly (Unrelated) 2 (4%) cases were reported as causing Adverse Drug Reactions.
As per the Hartwigs level of severity scale(13) 15 (60%) cases were found to have mild reaction and 5 (20%) cases each with moderate and severe reactions

According to Rawlins and Thomson(12) the type of reaction was classified as Type A (Predictable) with 21 (84%) cases and 4 (16%) cases with type B (Bizarre) reactions.

Looking for the Outcome of the patients, 15 (60%) cases were treated on OPD basis, and 10 (40%) cases required Hospitalization. Among them, 14 (56%) cases were recovered completely, 8 (32%) cases were in a state of recovering and 3 (12%) cases were continuing the treatment.

Most common route of adverse drug reactions was oral, followed by IV, IM, S/C, Topical and Inhalational routes.

Most patients recovered from adverse events taking suitable measures like complete stopping the offending agent, or were prescribed antihistamines and steroids.

**Discussion**

This study tried to find out the pattern of adverse drug reactions of drugs used in tertiary care hospital. The number of reports we received were 25 out of 30747 cases treated, which amounted to an incidence of 0.081% in our set up. In comparison with the study for search of adverse drug reactions in hospital patients in Embase and Medline found the occurrence of 2 – 27.7%, this can be considered as under reporting. It is a universal problem and many reasons are identified such as busy schedule of clinicians, lack of knowledge about the exact authority to report adverse drug reactions to, lack of incentives, reporting process being tedious and inadequate expertise. Our verbal discussions with clinicians revealed similar reasons for underreporting in our institution.

The demographic details of our study showed female gender predominance over males, which was similar to that reported in other studies found in the literature. This might be due to higher emotion quotient in females which makes them more sensitive to the pharmacological actions of medicines.

The most common category associated with adverse drug reactions was dermatology (44%). This finding is concurrent with the study carried out by Coelho et al. (2002) and Rajesh et al. (2008), but it differs from reports of Suh et al. (2000), where gastrointestinal manifestations had the highest rate. Of the dermatological reactions observed in the hospital, itching were seen in 45.45% and rashes in 36.36%.

The incidence rate of NSAIDS adverse drug reactions in this study was found to be comparatively high when compared to other drugs.

**Conclusion**

The reporting rate appeared to be low so there is need for increasing knowledge and awareness. Educational interventions like conducting CME and training programmes can improve the knowledge towards pharmacovigilance. However monitoring adverse drug reactions is an ongoing and continuing process. Since newer and newer drugs hit the market the need for pharmacovigillance grows more than ever before. Imparting knowledge and awareness of adverse drug reactions reporting of health care professionals would introduce the reporting culture among medical practitioners and increase the reporting rates of adverse drug reactions. Careful considerations involved in planning and monitoring of drug therapy will lead to preventions of adverse drug reactions.

**Acknowledgement:** We wish to thank all our clinicians, nursing staff, emergency staff of K M C H for kind support in carrying out this study. Also, we extend our thanks to Chairman, K M C H, and Principal for their constant and valid guidance, support, and encouragement.

**Ethical Clearance:** Institutional ethical clearance was obtained

**Conflict of Interest:** Nil

**Source of Funding** : Nil

**References**


Pattern of Homicidal Deaths at Raichur District Region – A Retrospective Study

Ravishankar M G¹, Sunil Kumar Kainoor², Suraj¹, S Sharmila³,
¹Final year Postgraduate Student, ²Assistant Professor, Department of Forensic Medicine and Toxicology, Raichur Institute of Medical Sciences, Raichur; Karnataka

Abstract

Background: It is a retrospective study carried out to find out the pattern of homicidal deaths in around the Raichur district region, among autopsies conducted at mortuary, RIMS, Raichur.

Materials and Methods: This is a two year retrospective study of autopsies conducted from January 2016 to December 2017 at Raichur Institute of Medical Sciences, Raichur. The objectives of the study were to know the pattern of homicidal deaths in and around Raichur district and to study the various socio-demographic factors influencing the homicidal deaths. Using a predefined and structured Performa, all the necessary details pertaining to the cases were collected from the inquest report and were analyzed.

Results: There were 757 total cases of autopsies conducted during the study period, of which there were 51 (6.73%) homicidal deaths. Male preponderance, 20-29 age group, blunt weapons - most commonly used, neck - commonest region of body involved constitutes 29.52%, most common cause of death - hemorrhage and shock 25.49%( 13) equally followed by Head injury, maximum homicide took place at the victim’s residence (58.82%), the most common motive behind the homicide was Infidelity (21.56%), maximum numbers of homicides were committed by Spouse (27.45%).

Conclusion: An attempt is made to know the socio demographic profile of the deceased so as to understand the sociological, economical, demographic and psychological aspects influencing homicidal deaths. The spurt in the homicidal deaths in our region may be attributed to the poor socio – economic condition, unemployment among young people, marital and family disputes, decreasing value based morality in the society, soft and sometimes toothless law enforcement agencies.

Key Words: Retrospective study, Homicidal deaths, Socio demographic analysis.

Introduction

Homicide means one human being causes death of another. Not all homicide is murder, as some killings are manslaughter, and some are lawful, such as when justified by an affirmative defense,

Like insanity or self defence.¹ A homicide is usually well-planned, therefore not normally witnessed.

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Killings remained the same v.i.z. lust for money, women and land. To commit murder, two elements (Mens–rea which means preplanning or afore thought and Actus Reus which means the actual execution).²

Homicide is defined as killing of one human being by another human being and is one of the leading causes of unnatural deaths.³ Unlawful killing of human being is murder (S.300 IPC). Culpable homicide cases may be amounting to murder (S.299 IPC) or not amounting to murder (S.304 IPC). Punishment of murder (S.302IPC) is death or imprisonment for life and also fine. The various patterns of homicidal deaths include assault by sharp weapon, blunt weapon, firearm, strangulation, homicidal hanging, smothering, drowning, burns, poisoning etc.⁴

DOI Number: 10.37506/ijfmt.v14i2.2791
Material and Method

This 2 year retrospective study was analyzed among autopsies conducted from January 2016 to December 2017 at Raichur Institute of Medical Sciences, Raichur. The objectives of the study were to know the pattern of homicidal deaths in and around Raichur city and to study the various socio-demographic factors influencing the homicidal deaths. Using a predefined and structured Performa, all the necessary details pertaining to the cases were collected from the inquest report- which includes police enquiry report, witness statement and relative statement, findings of post-mortem examination report, hospital case sheet extracts, histo-pathological examination report, toxicological (chemical) analysis report, crime scene photographs and was then tabulated to Microsoft Excel sheet 2007 for analysis and results were explained in number of cases and percentage. Prior to the study, ethical committee clearance was obtained from Institutional Ethical committee.

Results

There were 757 total cases of autopsies conducted during the study period, of which there were 51 (6.73%) homicidal deaths. The maximum cases were observed among age group 20-29 years (31%), followed by 30-39 years (21%), 60+ years (13.72%), 40-49 years (11.76%), 50-59 years and 0-9 years (7.84%) . The least number of cases were observed among the age group 10-19 years (6%).

More number of deaths were observed among males i.e., 51% (n=26), on cross tabulating age group with gender wise distribution it was observed that more number of deaths among males (23.04%) was seen in the age group 20-29 years and 32% of female deaths in the age group 40-49 years. (Table 1)

Table 1: Age group v/s Gender distribution

<table>
<thead>
<tr>
<th>Age group</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
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<td>3</td>
<td>4</td>
<td>7.8</td>
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<td>3</td>
<td>6</td>
<td>11.8</td>
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<tr>
<td>20-29 yrs</td>
<td>6</td>
<td>4</td>
<td>10</td>
<td>19.6</td>
</tr>
<tr>
<td>30-39 yrs</td>
<td>5</td>
<td>4</td>
<td>9</td>
<td>17.6</td>
</tr>
<tr>
<td>40-49 yrs</td>
<td>3</td>
<td>8</td>
<td>11</td>
<td>21.6</td>
</tr>
<tr>
<td>50-59 yrs</td>
<td>5</td>
<td>1</td>
<td>6</td>
<td>11.8</td>
</tr>
<tr>
<td>&gt;60 yrs</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>9.8</td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>25</td>
<td>51</td>
<td>100</td>
</tr>
</tbody>
</table>

Most number of cases were observed during the winter season (42%) i.e., November to February (n=21) followed by summer season (33%) i.e., March to June and Monsoon (25%) i.e., July to October. Most deaths occurred instantaneously i.e., 58.82% (n=30), followed by within 12 hrs following assault (19.61%) and between 12-24 hours in 5.88% cases.

In our study the more number of deaths were due to assault by blunt force (37%), followed by asphyxial deaths (25% - which include throttling, smothering, drowning and poisoning cases), burn injuries (18%), no deaths due to firearm injuries were observed. (Table 2)

Table 2: Distribution of cases on types of injuries observed

<table>
<thead>
<tr>
<th>Type of injury</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asphyxial deaths</td>
<td>13</td>
<td>25%</td>
</tr>
<tr>
<td>Firearm wound</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Stab wound</td>
<td>2</td>
<td>4%</td>
</tr>
<tr>
<td>Incised wound</td>
<td>4</td>
<td>8%</td>
</tr>
<tr>
<td>Chop wound</td>
<td>4</td>
<td>8%</td>
</tr>
<tr>
<td>Burns wound</td>
<td>9</td>
<td>18%</td>
</tr>
<tr>
<td>Blunt force wound</td>
<td>19</td>
<td>37%</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>100%</td>
</tr>
</tbody>
</table>
Lungs are the most common organs to sustain injuries (33%), followed by brain (25%), no injuries to kidneys and spleen observed in our study. (Table 3)

<table>
<thead>
<tr>
<th>Internal organs</th>
<th>Number of cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lungs</td>
<td>17</td>
<td>33%</td>
</tr>
<tr>
<td>Brain</td>
<td>13</td>
<td>25%</td>
</tr>
<tr>
<td>Stomach and intestine</td>
<td>4</td>
<td>8%</td>
</tr>
<tr>
<td>Heart</td>
<td>2</td>
<td>4%</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>2</td>
<td>4%</td>
</tr>
<tr>
<td>Testes</td>
<td>2</td>
<td>4%</td>
</tr>
<tr>
<td>Liver</td>
<td>1</td>
<td>2%</td>
</tr>
<tr>
<td>Multiple organs</td>
<td>1</td>
<td>2%</td>
</tr>
<tr>
<td>Kidney</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Spleen</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Misc</td>
<td>9</td>
<td>18%</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>100%</td>
</tr>
</tbody>
</table>

Most number of assaults involved neck region (30%), followed by head and face (23%), multiple regions (21%) and least injuries on extremities (2%). (Table 4)

<table>
<thead>
<tr>
<th>Region of body</th>
<th>Number of cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neck</td>
<td>15</td>
<td>30%</td>
</tr>
<tr>
<td>Head and face</td>
<td>12</td>
<td>23%</td>
</tr>
<tr>
<td>Multiple regions</td>
<td>11</td>
<td>21%</td>
</tr>
<tr>
<td>Abdomen</td>
<td>6</td>
<td>12%</td>
</tr>
</tbody>
</table>

In our study more than half of the assault occurred in home (59%), the next most common was work place (20%) while 16% of assaults occurred in street.

In our study, a proper history or reason for homicide was uncertain in 13 cases (25%), followed by infidelity (22%), revenge (15%), heated arguments (14%) and 10% financial conflicts. (Table 5)

<table>
<thead>
<tr>
<th>Number of cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unknown</td>
<td>13</td>
</tr>
<tr>
<td>Mental illness</td>
<td>1</td>
</tr>
<tr>
<td>Dowry</td>
<td>2</td>
</tr>
<tr>
<td>Property disputes</td>
<td>4</td>
</tr>
<tr>
<td>Financial conflict</td>
<td>5</td>
</tr>
<tr>
<td>Argument</td>
<td>7</td>
</tr>
<tr>
<td>Revenge</td>
<td>8</td>
</tr>
<tr>
<td>Infidelity</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
</tr>
</tbody>
</table>

Most number of assaults were by spouses (28%), followed by acquaintance (21%), relatives (21%), unknown assailants (14%) and parents and strangers in 8% cases each. Most common cause of death in our study is hemorrhage & shock and head injury each being 25%, followed by asphyxia (18%), septicemia (12%) and hypovolemic shock in 8% cases.

**Discussion**

In our study duration, (2 yrs) there were totally 757 cases autopsied of these 51 cases (6.73%) are homicidal deaths. In a similar study by Courtnee clark et al with study duration being 5 years the total percentage of homicidal deaths are 5.32%. Similar studies by others
showed lesser proportion of homicidal deaths i.e., Shailesh Jhaveri et al6 - 2.34% (3 yrs), Dr. Basappa S. Hugar et al2 4.32% (3yrs) and Ashok K. Rastogi et al7 4.25% (1 yr).

Majority victims in our study were males 50.98%, the results are in similarity with other studies conducted by Dr. Basappa S. Hugar et al2, Shailesh Jhaveri et al6, Courtnee clark et al5 and Ashok K. Rastogi et al7.

The most common age group to suffer in our study is 20-29 years (31.37 %), the same results are observed in various other authors like Dr. Basappa S. Hugar et al2, Shailesh Jhaveri et al6, Courtnee clark et al5 except Ashok K. Rastogi et al7 - 18-40 yrs.

In our study most cases are reported during the winter season 41.17% i.e., November to February (Nov-Feb), while in most other similar studies by Dr. Basappa S. Hugar et al2, Shailesh Jhaveri et al6, Courtnee clark et al5 and Ashok K. Rastogi et al7 the most number of cases are reported during warmer climate (Summer).

Warren et al. (1981), who found homicide to have a seasonal pattern that changes from year to year. That is, a “peak month” in some years is a “trough month” in other years. The authors conclude that homicide is seasonal, but inconsistent.

Blunt weapons being the most common weapon used in our study (37.25%) and the same results observed by Shailesh Jhaveri et al6, Courtnee clark et al5 and Ashok K. Rastogi et al7 while study by Dr. Basappa S. Hugar et al2 the sharp weapons are the most common weapons of offence.

In our study the most common region of body involved sustaining injuries is neck (29.52%), the same results observed in other studies by Dr. Basappa S. Hugar et al2, Courtnee clark et al5 and Ashok K. Rastogi et al7. While in a study by Shailesh Jhaveri et al6 multiple body structures involvement was common.

In our study both Shock and Hemorrhage (25%) & head injury (25%) are the most common cause of death, similarly in a study by Ashok K. Rastogi et al7 shock and hemorrhage 46.34% followed by asphyxia 20.73% was observed. In another study by Shailesh Jhaveri et al6 head injury (26.42%) is the most common cause of death.

Victim residence is the commonest site of occurrence of crime in our study (59%), the same observation was made by Shailesh Jhaveri et al6, Courtnee clark et al5 and Ashok K.Rastogi et al7.

**Conclusion**

The present study pattern of homicidal deaths in the Raichur district region has provided a number of revealing information about homicidal deaths. There has been a steady increasing trend in the homicidal deaths in our area. The spurt in the homicidal deaths may be attributed to the poor socio-economic condition, unemployment among young people, marital and family disputes, decreasing value based morality in the society, soft and sometimes toothless law enforcement agencies.

The government and society should identify the various social, economical, moral and law enforcement agencies problems that are directly or indirectly leading to the rise in the incidence of homicidal deaths and should address it through proper agency or department.

**Conflict of Intrest – None**

**Sources of Funding – Self**

**Ethical Clearance – Institutional Ethical Clearance Taken**

**References**


Chronic Alcoholism and Drug Abuse Behavior: A Menace to Society- A Research Study

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Abstract

Background: The present study was conducted to assess chronic alcoholism and drug menace in adults.

Materials & Method: The present study was conducted on 1026 subjects which were screened in the department. A questionnaire was given to all and response was recorded.

Results: Out of 1026 subjects, male were 650 and female were 376. 340 male and 48 female were alcoholic and 126 male and 15 female were using drugs. The difference was significant (P< 0.05). 210 subjects were below 30 years of age and 319 were above 30 years of age, living standard was low in 410 and medium in 119, education standard was upto high school in 290 and graduation in 239, 380 subjects were married and 149 were unmarried. The difference was significant (P< 0.05).

Conclusion: Authors found that age more than 30 years, low living standard and low education level were risk factors for alcoholism and drug usage among adults.

Key words: Alcoholism, Drug, Education

Introduction

In the recent years there has been rapid proliferation of city bars and nightclubs and people are fast shedding their inhibitions about alcohol as a lifestyle choice.1 This has led to fears of an undocumented rise in alcohol abuse not only among poorer classes but also in other sections of the society.2 Having recognized the problem, the ministry of health has called for a policy that will regulate sales and the pricing of alcohol which many experts believe, may not be enough to curb the problem. The increasing use of alcohol and its drink-related problems has already emerged as a major public health concern in India and which needs to be addressed. A large majority of male drinkers meet criteria for hazardous alcohol use, defined as patterns of use that increase risk for harmful consequences for the user or others. Data from different Indian states indicate that 35% to 65% of all current drinkers meet criteria for hazardous alcohol use.3

Illegal drug economy plays a prominent role in national decisions made. Fortunately, cost-effective measures targeting drug abuse treatment are available. This fact increases motivation to conduct studies in order to improve interventions compatible with cultural issues.

Various countries have taken measures at society level to change knowledge and attitude of the society toward illegal drugs. These interventions aimed to prevent illegal drug abuse. Usually, focus of these interventions was teenagers and the youth, as these age groups are at more risk of drug abuse due to less information regarding this problem.4 The present study was conducted to assess chronic alcoholism and drug menace in adults.

Materials & Method

The present study was conducted in the department of Community medicine. It comprised of 1026 subjects which were screened in the department. All were informed regarding the study and written consent was obtained. Ethical clearance was obtained prior to the study.

Data such as name, age, gender etc. was obtained. A questionnaire was given to all and response was recorded. Results thus obtained were subjected to statistical analysis. P value less than 0.05 was considered significant.
Results

Table I Distribution of subjects

<table>
<thead>
<tr>
<th>Gender</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>650</td>
<td>376</td>
</tr>
</tbody>
</table>

Table I shows that out of 1026 subjects, male were 650 and female were 376.

Table II Prevalence of alcoholism and drug usage

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Male (650)</th>
<th>Female (376)</th>
<th>Total</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcoholism</td>
<td>340</td>
<td>48</td>
<td>388</td>
<td>0.01</td>
</tr>
<tr>
<td>Drug usage</td>
<td>126</td>
<td>15</td>
<td>141</td>
<td>0.02</td>
</tr>
<tr>
<td>Total</td>
<td>466</td>
<td>63</td>
<td>529</td>
<td></td>
</tr>
</tbody>
</table>

Table II shows that 340 male and 48 female were alcoholic and 126 male and 15 female were using drugs. The difference was significant (P< 0.05).

Table III Risk factors for alcoholism and drug usage

<table>
<thead>
<tr>
<th>Variant</th>
<th>Parameters</th>
<th>Number</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>&lt;30 years</td>
<td>210</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>&gt;30 years</td>
<td>319</td>
<td></td>
</tr>
<tr>
<td>Living standard</td>
<td>Low</td>
<td>410</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>119</td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td>upto high School</td>
<td>290</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>Graduation</td>
<td>239</td>
<td></td>
</tr>
<tr>
<td>Marital status</td>
<td>Married</td>
<td>380</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Unmarried</td>
<td>149</td>
<td></td>
</tr>
</tbody>
</table>

Table III shows that 210 subjects were below 30 years of age and 319 were above 30 years of age, living standard was low in 410 and medium in 119, education standard was upto high school in 290 and graduation in 239, 380 subjects were married and 149 were unmarried. The difference was significant (P< 0.05).

Discussion

Alcoholism does not only impacts the drinker but also their families and communities and making things worse, it makes it more critical to assess for prevention and intervention efforts. Despite the public health crisis and harmful consequences alcoholism represents, there is inadequate recognition of alcohol misuse as a public health issue in India. Information on screening measures is critical for prevention and early intervention efforts.

Drug abuse, as a psychological-social-biologic issue, in adolescents is one of the most critical issues for countries nowadays. This problem involves new chemical drugs (e.g. crack and ecstasy) besides traditional ones (e.g. opium and marijuana). In Iran, even though there is no official report about drug abuse, clinical observations in drug addiction consultation centers and medical centers for drug addiction rehabilitation show that addiction to new psychoactive drugs in adolescents has a high prevalence. Nowadays, instead of traditional single-factor approach to the etiology of drug abuse, multifactorial and interactive causal approaches are considered as the etiology of drug abuse. The latter factors assess related factors to drug abuse.

It has been noted in different studies that drug abuse is affected by knowledge and attitude toward drugs. To change a behavior, at first knowledge and attitude toward that particular behavior should be corrected. Iran has more than 15 million adolescent and young people and is considered the youngest country in the world. There are few studies in this age group about their knowledge and attitude toward drug abuse, while attention to these views can help them guide to safer behaviors. The present study was conducted to assess chronic alcoholism and drug menace in adults.

In present study out of 1026 subjects, male were 650 and female were 376. We found that 340 male and 48 female were alcoholic and 126 male and 15 female were using drugs. The difference was significant (P< 0.05).

Khushabi et al included 157 adult male in the study. The mean age of the study participants was 37.20
years. The prevalence of alcoholism among the study participants was 35.7%. Among them only 4.5% who presented with symptoms of chronic alcoholism had taken treatment. Reasons for not taking treatment for alcoholism among study population were mainly due to their family problems (55.2%).

We found that 210 subjects were below 30 years of age and 319 were above 30 years of age, living standard was low in 410 and medium in 119, education standard was upto high school in 290 and graduation in 239, 380 subjects were married and 149 were unmarried. Farhadinasab et al\textsuperscript{10} found current use of alcohol to vary between 19.6% and 27.8% amongst the 50+ age group. Community survey carried out in Mumbai, which was restricted to males aged 45 years or over had found the use prevalence decreased rather rapidly in higher age groups (from 21.5% in the 55–59 age group to 5.7% in the 85+ age group). Alcohol consumption was 51.43% among adult males aged 30 years and below and 31% among the 30 years and above age group which was found to be statistically significant (p-0.027).

**Conclusion**

Authors found that age more than 30 years, low living standard and low education level were risk factors for alcoholism and drug usage among adults.

**Conflicts of Interest:** The author declare that there is no conflict of interest regarding the publication of this paper.

**Source of Funding:** Self

**Ethical Clearance:** Ethical clearance has been taken from Institutional Ethical Committee

**References**


Role of Physiotherapy on Quality of Life in Stroke Survivors – A Systematic Review

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Abstract

Background: Stroke is one of the most common disease with lots of impairments and disabilities. Prevalence of stroke is increasing day by day globally. Young stroke is becoming very common. The overall functional capacity of the individual is hampered where the prognosis is question mark. It largely depends upon site, stage, duration of lesion. The overall quality of function is deteriorated. Patients live their lives with dependency. According to previous studies, role of various health care professionals have been analyzed and studied. Physiotherapy is a branch where use of exercise and electrotherapy plays their role in rehabilitating patients with stroke

Aim: To study the role of physiotherapy on quality of life in stroke survivors.

Methodology: A systematic narrative review was conducted to find out the efficacy of physiotherapy treatment on quality of life in stroke survivors.

Conclusion: Stroke has complex process of recovery. Stroke survivors are left with disabilities. It causes depression in patients as well as their family members. Physiotherapy has a significant role in improving impairments and disabilities. This in turn helps in improving quality of life

Keywords: Physiotherapy, Stroke, Quality of life, Systematic narrative review.

Introduction

The Constitution of the World Health Organization (WHO) defines health as “A state of complete physical, mental, and social well-being not merely the absence of disease.”

It involves accessing health not only in terms of effects of health care, indication and severity of diseases but also focuses on well being by measuring the quality of life related to health care. WHO defines Quality of Life as an individual’s perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns. It is largely affected by individual’s psychological beliefs, physical health and social involvement. It serves as a reference against which an individual or society can measure the different domains of one’s own life. It is the general well-being of individuals and societies, outlining negative and positive features of life. It observes life satisfaction, including everything from physical health, family, education, employment, wealth, safety, security to freedom, religious beliefs, and the environment.

Quality of life and general health has various domains. Broadly they include physical health, psychological, level of independence, social relations, spirituality/ religion/ personal beliefs and environmental factors.

Quality of life is largely affected by type of food consumed, education, family life, emotional security, opportunities for activities etc. Health is one of the major issues that affects a person's quality of life.

Stroke and quality of life:

In 1970, the World Health Organization defined stroke as ‘rapidly developed clinical signs of focal (or global) disturbance of cerebral function, lasting more than 24 hours or leading to death, with no apparent cause
other than of vascular origin.2

Globally, cerebrovascular accident (stroke) is the second leading cause of death and the third leading cause of disability.3 The ratio of stroke is largely based in low and middle income countries. Death and disability is very common after stroke. In past few decades the ratio of stroke is increased in these counties as compared to high income countries. Young strokes are very common nowadays with hemorrhagic type overtaking the ischemic. According to the World Health Organization, 15 million people suffer stroke worldwide each year. Of these, 5 million die and another 5 million are permanently disabled. High blood pressure contributes to more than 12.7 million strokes worldwide. In developed countries, the incidence of stroke is declining, largely due to efforts to lower blood pressure and reduce smoking. However, the overall rate of stroke remains high due to the aging of the population. Almost half of stroke-related mortality may be attributable to modifiable risk factors (i.e. hypertension, diabetes, dietary risks, impaired glucose intolerance, obesity, smoking, air pollution, alcohol use, hypercholesterolemia, and physical inactivity), which are mostly the outcome of poor clinical management, limited access to health care, and late detection of underlying risk factors.4

Studies have shown that the Quality of life in stroke patients were decreased.5 Even with progress in treatment approaches for stroke and its prevalence ratio, the quality of life and social-related events caused by stroke has received limited attention. Stroke is an medical emergency which puts burden on medical care services as well as personal where loss of productivity in terms of activities and quality of living is compromised. Stroke presents with both structural and functional impairments which largely affects the physical performance. Motor and cognitive impairments are common. Gait, balance, limb function, psychological issues contribute to decline in quality of work.

Variety of treatment approaches is available for management. Medicine, physiotherapy, ayurveda, homeopathy etc has its own contribution in reversing the loss and improving functional gain.

Anxiety is more important in determining health related quality of life (HQOL) than depression.6 Even alteration in upper extremity functions adds to impaired quality of function.

**Physiotherapy in Stroke:**

Physiotherapy is an established field in stroke rehabilitation but uncertainties remain about the most appropriate intensity of therapy input. Stroke tends to result in a wide range of disabilities which have been shown to benefit from rehabilitation, in particular physiotherapy. Varieties of approaches are used in order to regain functional independence in trunk along with upper and lower extremities. Most of the members of the therapeutic team in stroke rehabilitation take the effectiveness of physical treatments after stroke for granted. The evidence available today suggests that it does not matter which form of treatment is chosen and that any of the available approaches will improve the patient’s functional status. In other words, if an optimal treatment exists, we have, so far, failed to identify it. Until further evidence emerges, we should therefore select therapies that are most cost-effective and that can be given to the largest number of patients.7 Physiotherapists play an inherent role in the multidisciplinary palliative care team emphasizing on improving function and quality of life in patients who are deemed to require physical and functional dimensions of care.8 It is important to analyze the role of physiotherapy in rehabilitating stroke and understanding its importance on quality of life.

**Physical therapy techniques:**

Conventional exercises are traditionally practiced throughout the world. Its role in establishing upper limb control has been found to be effective. Biofeedback has been shown to have better results in both acute and chronic stroke patients along with routine exercises.8

**Exercise therapy:**

Consists of passive movement, assisted movements, active movement, assisted-resisted active movements, and resisted movement. The techniques are to be applied in anatomical planes or as functional movement direction. These techniques can be performed on land or in water. The latter is termed as “hydrotherapy”. Best examples of therapeutic exercise techniques are relaxation, massage, suspension exercise, muscle-education, progressive resisted exercise, floor aerobics, active mobility exercises, mobilization and stabilization exercise, proprioceptive neuromuscular facilitation (facilitation and inhibition techniques); breathing exercise; postural training; work simulation, work conditioning and work hardening; graded activity program and cognitive-behavioral training.10 In recent times many neurophysiologic approaches have come up
which work exactly at the site of lesion. They work on principle of neuronal plasticity thereby having a huge impact on quality of life in stroke survivors.

**Neurophysiological approaches:**

**(Bobath concept):**

Bobath concept, also known as neuro developmental treatment, is a widely used approach in the rehabilitation of hemiparetic subjects in many countries. Bobath therapy has shown to have better functional outcome. It works on principles of Normal postural alignment, Inhibition versus facilitation, Assessment versus treatment.

**(Constrained induced movement therapy):**

Original form of CIMT contains three components (1) Intensive graded practice of the paretic upper limb aimed at enhancing task-specific use of the affected limb for up to 6 hours a day for 2 weeks (i.e., shaping) (2) constraining or FU of the non-paretic upper limb with a mitt to promote the use of the more impaired limb during 90% of the waking hours; and (3) adherence-enhancing behavioral methods designed to transfer the gains obtained in the clinical setting or laboratory to the patients’ real-world environment (i.e., transfer package).

A total of 24 patients were randomized to constraint-induced movement therapy or Bobath Concept group. Main measures were the Motor Activity Log-28, the Wolf Motor Function Test, the Motor Evaluation Scale for Arm in Stroke Patients and the Functional Independence Measure. Constraint-induced movement therapy and the Bobath Concept have similar efficiencies in improving functional ability, speed and quality of movement in the paretic arm among stroke patients with a high level of function. Constraint-induced movement therapy seems to be slightly more efficient than the Bobath Concept in improving the amount and quality of affected arm use.

**(Proprioceptive neuromuscular facilitation):**

Pelvic proprioceptive neuromuscular facilitation (PNF) helps to improve control of pelvis which is a key point for maintaining trunk control, gait and balance. PNF has shown to establish significant changes in motor function and functionality after training, suggesting that this program can be useful for rehabilitation of chronic stroke survivors.

**(Motor relearning programme):**

MRP works on the concept of analysis, finding the missing component, practicing it and transference of training. Task related training has shown to improve walking performance post stroke. It works on concept of relearning phenomenon thereby enhancing neuronal plasticity.

**(Roods approach):**

For every motor output proper and sequential sensory input is required. Roods approach works on this concept through sequence of primitive reflexes and milestones in order to achieve motor output. Various inhibitory and facilitatory techniques are used in order to regain motor control.

**(Brunnstrom technique):**

The predominant basis of the Brunnstrom approach is the use of reflexes to develop movement behaviour through (1) sensory stimulation to inhibit spasticity and (2) functional retraining to enhance movement control. A strong functional emphasis remains a feature of the Brunnstrom approach as a therapeutic tool for neurological dysfunction.

**(Electrotherapy):**

It is a branch of physiotherapy where low, medium and high frequency currents are used to gain therapeutic effects in various disorders.

**(Electrical modalities):**

Various electrotherapy modalities are been routinely used in treatment of impairments secondary to stroke. Reducing Pain, improving sensory input, maintaining muscular properties are the primary uses. Electrical stimulation, hot moist packs and exercises have shown to improve shoulder subluxation in stroke which is a main barrier for improving upper extremity functions.

**(Functional electrical stimulation):**

Functional electrical stimulation (FES) is a treatment that applies small electrical charges to a muscle that has become paralysed or weakened, due to damage in your brain or spinal cord. Research on its use in stroke for both foot drop and to assist finger movements, was first published in the late 1970s. In the mid-1980s, a group based in Salisbury in the UK started to look at using FES. Originally their work was in people with spinal
cord injuries; from this initial work they went on to develop devices for people with MS in the early 1990s and it continues to be used today. Electrical stimulation of the nerves result in contraction of muscles supplied by them. This technique can be used to improve the muscle strength, control the movements, bowel, bladder and sexual functions, maintenance of posture, standing and walking. FES is becoming popular in the treatment of shoulder subluxation, spasticity and weakness of upper and lower limbs in hemiplegic patients.

**EMG and biofeedback:**

This is a technique by which subject is made aware of activity of muscles for better self regulation of the motor functions. Electromyographic biofeedback (EMG-BFB) is a technique that is believed to have additional benefit when used with standard physiotherapy for the recovery of motor function in stroke patients. EMG biofeedback helps to recall anagrams using the cues. Mentally subject improves the performance by knowing the results of previous activity.

**Activity of daily living training:**

Individuals with stroke have difficulty in gaining functional independence in order to perform their activities of daily living. Physiotherapy works with a primary goal of achieving it. Spastic muscles make it very difficult to achieve voluntary control. Variety of interventions have been tried out and have shown to have significant impact on achieving positive results. Task-oriented training resulted in improved hand function and activities of daily living in stroke patients. Therapeutic gymnasium consists of instrumental training for achieving functional mobility in upper and lower extremity. Super rider, multiple gym exerciser, wall ladder, shoulder wheel, static bicycle, parallel bar with mirror, activity of daily living training table are routinely used to improve quality of function in them.

**Bladder dysfunction:**

The incidence of incontinence depends on the interval between the stroke and evaluation. The reported figures are: 1st week - 60%, 6th week - 42%, and 12th week - 29%. The bladder dysfunction in a stroke patient may be due to inability to communicate, immobility, dementia or neurogenic bladder dysfunction. Valsalva and credes maneuver are common approaches used in practice to regain control. Bladder training program can be achieved by Intermittent catheterization and timed voiding program.

**Preventive complications:**

Stroke, can come up with number of complications like shoulder pain, pressure sores, DVT etc. shoulder pain interferes sleep and therapy. Physiotherapy should be thought of as a first-line management for patients with subacromial impingement syndrome. Both exercises and electrical agents play role is reducing pain. Positioning and bed mobility exercises helps to reduce pressure sores. Aim of positioning is to prevent development of abnormal posture, spasticity and contractures. Normal anatomical alignment of head, trunk and limbs should be maintained. I125 labelled fibrinogen leg scans of patients with hemiplegia have shown evidence of DVT in 30-75% during the first week after stroke. Mobilization of patient as early as possible reduces the chances of blood clot formation and risks of DVT.

**Conclusion**

Stroke has complex process of recovery. Stroke survivors are left with disabilities. It causes depression in patients as well as their family members. Physiotherapy has a significant role in improving impairments and disabilities. This in turn helps in improving quality of life.

**Conflict of Interest:** No conflicts of interest

**Source of Funding:** KIMSDU Karad

**Ethical Clearance:** From institutional ethical committee of KIMSDU karad

**References**


Effect of Goal Oriented Exercises Versus Combined Physiotherapy Intervention on Functional Independence in Subject With Traumatic Brain Injury

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¹Assistant Professor; ²Associate Professor, Department of Neurosciences, Krishna College of Physiotherapy, Krishna Institute of Medical Sciences Deemed to be University, Karad, Maharashtra India

Abstract

Background- Traumatic brain injury is a devastating neurological disorder and a leading cause of death and acquired disability in India. Traumatic brain injury leads the damage to the brain that result in impairment in physical, cognitive, speech/language, daily activities and quality of life and behavioural functioning. These impairments have direct impact on the individuals functional outcome after traumatic brain injury. In rural areas the health care delivery system is still an upcoming area. There is scarcity in rehabilitation units to provide health care. Many researches has been done on motor and balance impairment but there is lack of researches done on goal oriented exercises and combined physiotherapy intervention on functional independence. In this study we are given goal oriented exercises mainly concentrate on problem list of patients and combined physiotherapy intervention given combining all the exercises and give it to the patients. Indeed this made us to study the effect of Goal Oriented Exercises Versus Combined Physiotherapy Intervention on Functional Independence in Subject with Traumatic Brain Injury. Objectives- 1. To find out effect of Goal oriented exercises On functional independence In subjects with traumatic brain injury. 2. To find out effect of Combined Physiotherapy Intervention On functional independence In subject with traumatic brain injury. Method- 44 subjects diagnosed with Traumatic Brain Injury were included in this study. Subjects were divided into two different groups. Group A was Given Goal Oriented Exercises and group B was given combined Physiotherapy intervention. These subjects were allocated by convenient sampling method. During Pre and post treatment assessment functional mobility were assessed by outcome measures Functional Independence Measure and Barthel Index. These outcome measures were analysed. Result- Pre and Post functional independence and mobility was analysed by using paired and unpaired t test. Data analysis showed significance for FIM and Barthel Index (p value less than 0.001) for both the groups but comparative to group A (Goal oriented Exercises), group B (Combined Physiotherapy Intervention) is more effective. Conclusion- Goal oriented Exercises and Combined Physiotherapy Intervention is effective in improving the functional independence and mobility in the post Traumatic Brain Injury individuals but comparative to Goal oriented Exercises, Combined Physiotherapy Intervention is more effective.

Keywords- Traumatic brain injury, Functional Independence Measure, Physiotherapy Intervention.

Introduction

Traumatic brain injury is defined as an alteration in brain function caused by an external force. Brain tissue damage can be of two type: primary injury and secondary injury. Primary injury occurs due to direct trauma to the parenchyma. Secondary injury results from cascade of biochemical, cellular, and molecular events that occur due to an initial injury and injury related hypoxia, elevated intracranial pressure and oedema. (1) There is
open head injury and close head injury, open head injury is associated with skull fracture and close head injury occurs in isolation without skull fracture. Blood supply of brain may also get indirectly affected due to the injury of face and neck. Most TBI occurs due to road traffic accident (RTA). The severity of brain injury may be categorised as mild, moderate and severe head injury. (2)

In Mild traumatic brain injury there is minimal damage to the neuroanatomical structures with slight or no permanent impairment. Glasgow coma scale score of more than 13 is defined as mild TBI. In moderate TBI GCS between 8 -13 and the post traumatic amnesia lasting between 1 to 24 hours. Severe TBI is GCS score less than 8. (2)

After TBI – state of consciousness, sensorimotor changes, cognitive, emotional and behavioural changes, attention, balance, dizziness and visual dysfunction, spasticity and post traumatic headache. (2)

Functional Independence is a common consequence of traumatic brain injury. To maintain a mobility, it is important that central nervous system receives and integrates a somatosensory information, which includes both the tactile and proprioceptive system. The receptors which carry tactile sensations are Merkels cells, Pacinian corpuscles, Meissners corpuscles and Ruffini endings. And they are found in feet and might play an important role in maintaining body stability and balance in upright stance. The proprioceptive receptors are muscle spindle and Golgi tendon organs which carry the sense of joint position and joint motion. The proprioceptive receptors in feet are sensitive to ankle rotation and can give information about balance. (3, 4) Stimulation of these receptors may improve balance control in individual with post TBI. The rhythmic auditory stimulation (RAS) also improves the balance and gait of post TBI patients by stimulating brain functions involved in movement, cognition, emotions and sensory perception also RAS helps in execution of movements and normalizing gait parameters. (5, 6, 7) Balance incorporates two vestibular reflexes- the vestibuloocular reflex (VOR) and the vestibulospinal reflex (VSR). To improve balance after TBI the vestibular balance rehabilitation therapy and visual treatments are commonly used. (8)

Postural impairment and Functional Independence are common consequences of TBI which may result in instability while performing day today activities like standing, walking, grasping, difficulty while coordinating eye-head movement and problem while focusing on target. The postural and coordination defect result from damage to cerebellum. (9)

The motor disability following TBI is a common, motor performance can be affected by damage to various loci in the nervous system which can cause reduction in muscle power and tonus disorder which can result in impaired balance. (6, 7) Focal or diffuse spasticity may appear following TBI. Time post injury is an important consideration as spontaneous neurological recovery may continue for 9- 15 months post injury. Motor impairment can also result from prolong immobilization and bed rest during acute period, prolonged immobility can cause effect on multiple body system that impact the motor function the most. Motor rehabilitation is essential in helping the patient re-establishing independence post TBI. (7, 8)

Examination of Functional Independence in TBI patients is done by using Functional Independence Measure Scale and Barthal Index(10, 11, 12) Initially many therapist concentrate on the motor component of patient to improve his independence in this study we are going to give Combined physiotherapy intervention for Functional Independence in TBI Individuals.

Material and Method

This was a study to find the “Effect of goal oriented exercises versus combined physiotherapy intervention on functional independence in subject with traumatic brain injury. The study was carried out in hospitals from Karad. An approval for the study was obtained from the protocol committee and ethical committee of KIMSDU. Individual were approach and those fulfilling the inclusive criteria were selected. The purpose was explained and written inform consent was taken prepared in accordance with the Helsinki Declaration from those who are willingly to participate. Total 44 individuals were taken. The inclusion criteria was TBI patients, Patients with mild and moderate TBI, Both male and females, Age- above 18 years, Patients with functionally Dependent. The exclusion criteria was individual who are unable to follow simple commands, unconscious patients, unstable vitals and candidates with any fracture of spine, lower and upper limb. Their Functional Independence was assessed by FIMS and Barthal Index. Then the candidates were divided in to two groups for group A goal oriented exercises and for group B Combined Physiotherapy Intervention are
given. This was given for 4 days per week for 6 weeks up to 45-60 minutes per session including rest time. The time of session increased as the week progresses, initially it took 30-35 minutes per session and later progressed to 45-60 minutes per session on 6th week.\(^{(13)}\). Later evaluation and interpretation was done using statistical analysis.

**Finding**

The data was analyzed using INSTAT software. Descriptive statistics were used to analyze for demographic data: Pre and post treatment protocol was analyzed by using paired and unpaired t test and p value <0.0001 was considered to be statistically significant.

**Within Group Comparison**

Within group comparison was done by applying ‘Paired t-test’ to pre and post training values of Functional independence measure.

**Table 1: Comparison of Pre and Post-treatment average with Functional Independence Measure**:-

<table>
<thead>
<tr>
<th>Group</th>
<th>Pre training Mean</th>
<th>Post training Mean</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>54.364</td>
<td>69.909</td>
<td>11.984</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Group B</td>
<td>55.955</td>
<td>59.545</td>
<td>10.021</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

**Table 2: Comparison of Pre and post-treatment average with Barthel Index**

<table>
<thead>
<tr>
<th>Group</th>
<th>Pre training Mean</th>
<th>Post training Mean</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>30.227</td>
<td>60</td>
<td>18.678</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Group B</td>
<td>31.364</td>
<td>36.818</td>
<td>17.390</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

**Between Group Comparison**

Between groups comparison was done by applying ‘unpaired t test’

**Table 3: Comparison of Pre and post-treatment average with Functional Independence Measure**:-

<table>
<thead>
<tr>
<th>Group</th>
<th>Group A</th>
<th>Group B</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre training Mean</td>
<td>54.364</td>
<td>56.500</td>
<td>0.7822</td>
<td>0.4385</td>
</tr>
<tr>
<td>Post training Mean</td>
<td>69.909</td>
<td>59.545</td>
<td>3.517</td>
<td>0.0011</td>
</tr>
</tbody>
</table>

**Table 4: Comparison of Pre and Post-treatment average with Barthel Index**

<table>
<thead>
<tr>
<th>Group</th>
<th>Group A</th>
<th>Group B</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre training Mean</td>
<td>30.227</td>
<td>37.045</td>
<td>2.817</td>
<td>0.0074</td>
</tr>
<tr>
<td>Post training Mean</td>
<td>60</td>
<td>36.818</td>
<td>6.795</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>
Discussion

The purpose of this study was to find out the effect of goal oriented exercises versus combined physiotherapy intervention on functional independence in subject with traumatic brain injury. About 40% of patients report Functional Dependence after TBI. In many research’s done before treatment was given mainly on motor component to improve balance in TBI patients. Many currently practicing physiotherapist mainly concentrate only on motor components of patients. In this study we have given Goal oriented exercises and Combined Physiotherapy Interventions.

Our study included total 44 post TBI candidates with Functional Dependence. Motor impairment is a common problem in post-TBI patients. This problem may persist for life time after TBI which can affect the Patients daily activities and his mobility. For this early management of patients in its acute stage plays an important role. Motor management alone with combined physiotherapy have a more effect in improving Functional Independence in post TBI patients. Vestibular apparatus has also been identified as sensory organ that controls sensations of balance and equilibrium in individual. In this study we found that candidates also had impairment in eye-head coordination movements which was improved by vestibular exercises which help in stabilizing gaze and eventually to improve the balance.

Motor disturbances following TBI includes reduction of power, tonus disorder (spasticity) and motor control disorganisation. Reduction of spasticity helps in improving ROM and functional activities. The static balance is improved by decreasing muscle tone of neck and upper trunk. Thus improving Functional Independence of an individuals.

Limitation of this study was that the study is done with small sample size and in limited geographical area.

Conclusion

Through this study, it is concluded that, Goal Oriented Exercises and combined physiotherapy intervention is effective in improving the functional independence and mobility in the post TBI individual but comparative to Goal oriented exercises, combined physiotherapy intervention is more effective.

Conflicts of Interest: There is no conflict of interest in this study.

Source of Funding

The study was funded by Krishna institute of medical sciences deemed to be university, Karad.

Ethical Clearance: This study has undergone ethical clearance through the university level ethical committee.

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The Effect of Structured Physiotherapy Exercise Programme on Lung Function in Industrial Workers

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Abstract

Context: Repeated exposure to dusty environment in an industry causes airway diseases which may affect pulmonary function over a period of time. Aim: The effects of structured physiotherapy exercises programme on lung function in sugarcane industrial workers. Study Design: An experimental study was conducted on 60 sugar factory workers at in and around Karad area. Purpose of the study: To find out the effect of structured physiotherapy exercises programme on lung function in industrial workers. Materials And Methodology: -60 workers of age group 20-40 from sugar industry participated in this study. They were bounded in single group. The subjects were scrutinized based on the inclusion criteria. All the subjects signed a consent form prior to participation in the study. Before receiving any intervention, the peak expiratory flow rate and 6 min walk test was taken and abdominal strength checked by manual muscle testing of abdominal muscle (graded abdominal muscle testing). After recording the pre-intervention outcome measure, the specific exercise protocol was given to the subjects which was included abdominal muscle exercises (graded abdominal muscle exercises) and 6 minute walk test as exercises purpose and diaphragmatic breathing exercises (10 repetition for 3 sets), segmental breathing exercises (10 repetition for 3 sets), incentive spirometry (20 repetition 5 sets). Post treatment outcome measure were performed for peak expiratory flow rate, abdominal muscle strength and 6 minute walk distance. Statistical analysis was done using paired test. Result: In this study pre-intervention peak expiratory flow rate was 290±58.251 and post-intervention peak expiratory flow rate was 307±60.914. In peak expiratory flow rate statistically extremely significant difference and increasing peak expiratory rate post intervention with (p<0.0001) with t=5.633 with 59 degree of freedom. The six-minute walk distance (6mwd) increased from 215 to 297 meter. In the study the pre intervention values of abdominal muscle strength (MMT or grades of abdominal muscle) was 1.63±0.7357 and post intervention abdominal muscle strength was 2.5±0.7249. In abdominal muscle strength statistically extremely significant difference and increases abdominal muscle strength with (p<0.0001) with t=11.851 with 59 degree of freedom. Conclusion: Thus, the above study concluded that a structured exercises programme showed a significant improvement clinically as well as statistically significant on the peak expiratory flow rate and 6-minute walk distance and abdominal muscle strength in the sugar industry workers. Hence it accepts the alternate hypothesis.

Keywords: PEFR, 6 minute walk distance, graded abdominal muscle exercises, Breathing exercises.

Introduction

Maharashtra is one of the largest sugar producers state in India. “Bagassosis” is a respiratory disease. Bagasse is a by-product of sugarcane crushing, size range from 0.5-3 microns are called as repairable dust, to which sugar factory workers are exposed by virtue of their occupation. Respiratory disease is today an important clinical problem for industrial workers. Significantly increased risk of respiratory morbidity
and mortality among industrial workers. Bagassosis, an interstitial lung disease, is a type of hypersensitivity pneumonitis attributed to exposure to moldy molasses. It reduced FVC, TLC, PEFR in bagassosis patients. Since 1970 very few pulmonary function studies were reported in this field until recently in 2008 a study in western Maharashtra reported decreasing FVC, FEV1, PEFR in occupationally exposed groups to bagasse as compared to non-exposed group. However, this study was conducted during the operational period of a sugar factory1,3,4,8,9.

**Peak Expiratory Flow Rate** 3,11,19

The peak expiratory flow (PEF), also called peak expiratory flow rate (PEFR) is a person’s maximum speed of expiration, as measured with a peak flow meter, a small, hand-held device used to monitor a person’s ability to breathe out air. It measures the airflow through the bronchi and thus the degree of obstruction in the airways.

Graded abdominal muscle exercises include2,12,20,21

Upper abdominal exercises-

Starting position-The subjects were instructed to lie in supine with the hips at 45 degree and knees at 90 degree and hand at sides. In all these activities subjects were instructed to keep the low back flat.

Grade1-subjects were asked to perform the curl ups by contracting abdominal muscles and then lifting the head off table with flexed knees.

Grade 2-the progression was made by lifting the shoulders until the top of scapulae lift from table, keeping the arms extended towards knees.

Grade 3- The next progression was done by lifting the shoulders until the scapulae clear table, keeping the arms horizontal.

Grade 4- The subjects were asked to progresses further by keeping the arms crossed over chest, until scapulae clear table.

Grade 5- The subjects were asked to progress the difficulty of the curl ups by having the subject change the arm position from horizontal and then to behind the neck, until scapulae clear table.

6 minute walk test provides simple, inexpensive, non-invasive method for assessing cardiac or pulmonary functional capacity. The 6-minute walk test measures the distance an individual is able to walk over 6 minutes. The goal is for individual to walk as far as possible in 6 minutes. The individual is allowed to self-pace and rest as needed as they traverse back and forth along a marked walking. The vitals should be noted prior as well as after the test. 6-minute walk test is used for measuring the response to medical interventions in patient with moderate to severe heart or lung diseases. 6-minute walk test has also been used as a one-time measure of functional status of patient as well as a predictor of morbidity and mortality5,6,10.

**PARTICIPANTS**

60 workers of age group 20-40 from sugar industry participated in this study. They were bounded in single group. The subjects were scrutinized based on the inclusion criteria. All the subjects signed a consent form prior to participation in the study. Before receiving any intervention, the peak expiratory flow rate and 6min walk test was taken and abdominal strength checked by manual muscle testing of abdominal muscle (graded abdominal muscle testing). After recording the pre-intervention outcome measure, The specific exercise protocol was given to the subjects which was included abdominal muscle exercises (graded abdominal muscle exercises)and 6 minute walk test as exercises purpose and diaphragmatic breathing exercises (10 repetition for 3 sets), segmental breathing exercises (10 repetition for 3 sets), incentive spirometry (20 repetition 5 sets), post treatment outcome measure were performed for peak expiratory flow rate,and abdominal muscle strength and 6 minute walk test distance. Statistical analysis was done using paired’ test.

**Outcome Measures:**

1. **Peak expiratory flow rate** 2,10,19: The peak expiratory flow (PEF), also called peak expiratory flow rate (PEFR) is a person’s maximum speed of expiration, as measured with a peak flow meter, a small, hand-held device used to monitor a person’s ability to breathe out air. It measures the airflow through the bronchi and thus the degree of obstruction in the airways.

2. **MMT for abdominal muscle** 2,20,21 -

Graded abdominal muscle exercises include

Upper abdominal exercises-

Starting position-The subjects were instructed to
lie in supine with the hips at 45 degree and knees at 90 degree and hand at sides. In all these activities subjects were instructed to keep the low back flat.

Grade 1-subjects were asked to perform the curl ups by contracting abdominal muscles and then lifting the head off table with flexed knees.

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Grade 5- The subjects were asked to progress the difficulty of the curl ups by having the subject change the arm position from horizontal and then to behind the neck, until scapulae clear table.

3. 6minute walk test provides simple, inexpensive, non-invasive method for assessing cardiac or pulmonary functional capacity. The 6-minute walk test measures the distance an individual is able to walk over 6 minutes. The goal is for individual to walk as far as possible in 6 minutes. The individual is allowed to self-pace and rest as needed as they traverse back and forth along a marked walking. The vitals should be noted prior as well as after the test. 6-minute walk test is used for measuring the response to medical interventions in patient with moderate to severe heart or lung diseases. 6-minute walk test has also been used as a onetime measure of functional status of patient as well as a predictor of morbidity and mortality.

Methodology

60 workers of age group 20-40 from sugar industry participated in this study. They were bounded in single group. The subjects were scrutinized based on the inclusion criteria. All the subjects signed a consent form prior to participation in the study. Before receiving any intervention, the peak expiratory flow rate and 6min walk test was taken and abdominal strength checked by manual muscle testing of abdominal muscle (graded abdominal muscle testing). After recording the pre-intervention outcome measure,The specific exercise protocol was given to the subjects which was included abdominal muscle exercises (graded abdominal muscle exercises)and 6 minute walk test as exercises purpose and diaphragmatic breathing exercises (10 repetition for 3 sets), segmental breathing exercises (10 repetition for 3 sets), incentive spirometry (20 repetition 5 sets).post treatment outcome measure were performed for peak expiratory flow rate, and abdominal muscle strength and 6 minute walk test distance. Statistical analysis was done using paired’ test.

Results

1) Peak Expiratory Flow Rate: The pre intervention peak expiratory flow rate value were 290 ± 58.251, whereas post intervention the value was 307.33 ± 60.914. the post intervention change in peak expiratory flow rate values showed statistically extremely significant. The “P” value is <0.0001 considered extremely significant. This was done using paired’ test. “t” value is t=5.633 with 59 degree of freedom.

2) Manual Muscle Testing:

The pre interventional MMT value was 1.63±0.7357 whereas post interventional the value was 2.5±0.7249. the post interventional change in value showed statistically extremely significant. The “P” value is <0.0001 considered extremely significant. This was doing using paired” t” test. the “t” value is t=11.851 with 59-degree freedom.

3) 6 minute walk test:

The pre interventional 6mwd value was 1.63±0.7357 whereas post interventional the value was 2.5±0.7249. the post interventional change in value showed statistically extremely significant. The “p” value is <0.0001 considered extremely significant. This was doing using paired” t” test. the “t” value is t=11.851 with 59-degree freedom.

1.Peak Expiratory Flow Rate -

Table 1: Mean and standard deviation of pefr

<table>
<thead>
<tr>
<th>Pefr</th>
<th>Pre Pefr</th>
<th>Post Pefr</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEAN</td>
<td>290</td>
<td>307.33</td>
</tr>
<tr>
<td>SD</td>
<td>58.251</td>
<td>60.914</td>
</tr>
</tbody>
</table>
2. Manual Muscle Testing:

**Table 2: Mean and SD of MMT**

<table>
<thead>
<tr>
<th>MMT</th>
<th>PRE MMT</th>
<th>POST MMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEAN</td>
<td>1.63</td>
<td>2.5</td>
</tr>
<tr>
<td>SD</td>
<td>0.7357</td>
<td>0.7249</td>
</tr>
</tbody>
</table>

3. 6 MINUTE WALK DISTANCE:

**TABLE 3: MEAN AND SD OF 6MWD**

<table>
<thead>
<tr>
<th>6MWD</th>
<th>Pre 6MWD</th>
<th>Post 6MWD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance</td>
<td>215</td>
<td>310</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>307.8±46.0</td>
<td>344.5±64.5</td>
</tr>
</tbody>
</table>

Discussion

Maharashtra is one of the largest sugar producers state in India. “Bagassosis” is a respiratory disease. Bagasse is a by-product of sugarcane crushing, size range from 0.5–3 microns are called as repairable dust, to which sugar factory workers are exposed by virtue of their occupation. Respiratory disease is today an important clinical problem for industrial workers. Significantly increased risk of respiratory morbidity and mortality among industrial workers. Bagassosis, an interstitial lung disease, is a type of hypersensitivity pneumonitis attributed to exposure to moldy molasses. It reduced FVC, TLC, PEFR in bagassosis patients. Since 1970 very few pulmonary function studies were reported in this field until recently in 2008 a study in western Maharashtra reported decreasing FVC, FEV1, PEFR in occupationally exposed groups to bagasse as compared to non-exposed group. However, this study was conducted during the operational period of a sugar factory.\textsuperscript{1}Nikhade N., Sharma P conducted a Study Of Pulmonary Function Test In this study Workers Of Sugar Factory, Pravaranagar, Maharashtra, in this study Significant reduction of FEV1 in Bagasse workers, Manufacturing department and Engineering department workers as compared with controls, indicated obstructive type of pulmonary abnormalities. Reduced FEV1 has earlier been reported by Bohadana et al\textsuperscript{23} showed that workers exposed to sugar dust in the sugar cubemanufacture workstation had significantly lower forced expiratory volume in 1s (FEV1) than the non-exposed ones. The Peak Expiratory Flow Rate (PEFR) was reduced in all the exposed workers in sub-occupational groups being higher in Bagasse workers followed by Manufacturing department. A highly insignificant decrease in PEFR was also reported from western Maharashtra by Patil S.N.\textsuperscript{3} PEFR is an index of expiratory airway resistance and is more effort dependent. The reduction in PEFR may involve the same mechanism already explained for obstructive lesion. In addition, the inflammatory reaction releases proteins from eosinophils which might be responsible for the hyper responsivenss of airways. In this study 60 sugar industrial workers of age 20-40 years in Karad area were been participated in the study. They were bounded in single group. The pre outcome measure was peak expiratory flow rate, abdominal strength and 6 minute walk distance. Peak expiratory flow rate measured by peak expiratory flow rate device, 6 minute walk distance was measured by 6min walk test abdominal muscle strength measured by grades of abdominal muscle. The specific exercise protocol was given to the subjects which was included abdominal muscle exercises (graded abdominal muscle exercises) and 6 minute walk test as exercises purpose and diaphragmatic breathing exercises (10 repetition for 3 sets), segmental breathing exercises (10 repetition for 3 sets), incentive spirometry (20 repetition 5 sets). Post treatment outcome measure were performed for peak expiratory flow rate, and abdominal muscle strength and 6 minute walk test distance. Statistical analysis was done using paired\textsuperscript{t} test. In this study pre-intervention peak expiratory flow rate was 290±58.251 and post-intervention peak expiratory flow rate was 307±60.914. In peak expiratory flow rate statistically extremely significant difference and increasing peak expiratory rate post intervention with (p<0.0001) with t=5.633 with 59 degree of freedom. The six-minute walk distance (6mwd) increased from 215 to 297 meter. In the study the pre intervention values of abdominal muscle strength (MMT or grades of abdominal muscle) was 1.63±0.7357 and post intervention abdominal muscle strength was 2.5±0.7249. In abdominal muscle strength statistically extremely significant difference and increases abdominal muscle strength with (p<0.0001) with t=11.851 with 59 degree of freedom. Thus, the above study concluded that a structured exercises programme showed a significant improvement clinically as well as statistically significant on the peak expiratory flow.
rate and 6 minute walk distance and abdominal muscle strength in the sugar industry workers. Hence it accepts the alternate hypothesis.

**Limitations:**

1. The sample size was small.
2. Limited literature review on industrial workers.
3. Limited literature review on peak expiratory flow rate.
4. Limited literature review on structured physiotherapy exercises programme on industrial workers.

**Conclusion**

Thus, the above study concluded that a structured exercise programme showed a significant improvement clinically as well as statistically on the peak expiratory flow rate, abdominal muscle strength and 6-minute walk distance in the sugar industry workers. hence it accepts the alternate hypothesis.

**Conflicts of Interest:** Nil

**Source of Funding:** Krishna Institute Of Medical Sciences Deemed University, Karad.

**Ethical Clearance:** Study approved by Institutional ethics committee of Krishna institute of medical sciences deemed to be university, Karad.

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Effect of Integrated Neuromuscular Inhibition Technique on Frozen Shoulder

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Abstract

Background: Frozen shoulder is a painful condition of the joint capsule leading to loss of range of motion of the joint. Due to pain and inability to move the involved shoulder, patients tend to avoid any movement of the involved arm. This leads to formation of Myofascial Trigger points and tender points in the muscles. This overall makes the prognosis more challenging as the focus is mostly on the joint. Integrated Neuromuscular Inhibition Technique is a soft tissue manipulation technique that has been proven to relieve trigger and tender points. Although proven to be efficiently effective in many disorders, this technique is not as such practiced as a common and regular approach by professionals. Thus, this technique was undertaken and experimented on Frozen Shoulder.

Objectives: Objectives of the study were to determine the effect of Integrated Neuromuscular Inhibition Technique in Frozen Shoulder and compare the effect of Integrated Neuromuscular Inhibition Technique with conventional treatment in frozen shoulder.

Material and Method: In this experimental study, 40 Frozen Shoulder subjects were assessed using Visual Analogue Scale (VAS), Goniometer for Range of Motion (ROM) and Shoulder Pain and Disability Index (SPADI). The subjects were divided into 2 groups, Group A included 20 subjects treated with Hot moist pack, Therapeutic Ultrasound, Exercises and Capsular Stretching and Sleeper stretch. Group B included 20 subjects treated with Hot moist pack, Therapeutic Ultrasound, Exercises and Capsular Stretching and Sleeper stretch and Integrated Neuromuscular Inhibition Technique.

Results: Within group statistical analysis of VAS, ROM and SPADI for both the groups were found to be extremely significant with a p value of <0.0001. On comparing between the two groups post treatment, there was extremely significant difference in Group B as compared to Group A regarding VAS (p=0.002), very significant difference for SPADI (p=0.0047), FLEXION ROM (p=0.0076) and ABDUCTION ROM (p=0.0026). But, there was no significant difference between the two groups regarding MEDIAL ROTATION ROM (p=0.1772) and LATERAL ROTATION ROM (p=0.7602).

Conclusion: The study concluded that Integrated Neuromuscular Inhibition Technique and Conventional Physiotherapy are both effective in the management of Frozen Shoulder. Also, Integrated Neuromuscular Inhibition Technique given with Conventional Treatment is significantly more effective as compared to conventional treatment alone on pain, range of motion and functional status of Shoulder and thus Alternate Hypothesis accepted.

Keyword: Frozen Shoulder, Integrated Neuromuscular Inhibition Technique, Pain, Muscle Release Intervention, Range of Motion.

Introduction

Frozen shoulder or adhesive capsulitis is a commonly occurring condition characterized by a capsular pathology associated with pain and progressive loss of passive and active movement. The incidence of Frozen Shoulder in General population is 2-5%, while
in patients with Diabetes Mellitus rises to 20-29% and is more common in females above 40 years of age.\textsuperscript{(1)}

This inflammatory condition that leads to fibrosis of the glenohumeral joint capsule is seen with gradually progressive stiffness and significant restriction of range of motion (typically external rotation).\textsuperscript{(2)} The causes for Frozen Shoulder have been classified according to two types namely Primary and Secondary Frozen Shoulder. While primary is idiopathic, secondary is ought to be due to conditions like Diabetes mellitus, Stroke, Thyroid disorder, Shoulder injury, Dupuytren’s disease, Parkinson disease, Cancer, Complex regional pain syndrome.\textsuperscript{(3)}

Subjects suffering with Frozen Shoulder often come up with symptoms of Pain usually Radiating to the deltoid insertion, nocturnal pain that interferes with sleep and restricted movement particularly abduction and external rotation.\textsuperscript{(4)} The condition is marked into four stages through its course starting from the first stage being An Acute Painful Stage, progressing to a Freezing Stage, followed by a Frozen Stage and lastly A Thawing Stage.\textsuperscript{(5,6,7)}

Diagnosis can be made on the basis of history and investigations. Other conditions causing a painful and stiff shoulder are ruled out, the affected shoulder is assessed to identify the stage of the disorder, Any History of previous trauma or surgery around shoulder is enquired. About 50% reduced range of motion may be seen. History of Cardiac or Neurosurgery, impaired consciousness and hemiparesis and other neurological conditions like Parkinson’s disease and brachial neuritis. Cervical spondylitis, Diabetes and thyroid disorders and a normal joint appearance in radiograph.\textsuperscript{(4)}

Integrated Neuromuscular Inhibition Technique is a combination of a few techniques in a stepwise application. It involves using the position of ease as part of a sequence which commences with location of a tender/trigger point, application of ischemic compression and introduction of positional release. After an appropriate length of time, during which the tissues are held in ease, the patient introduces an isometric contraction into the affected tissues for 5-7 seconds. After this, the local tissues housing the trigger point is stretched.\textsuperscript{(8)}

The rationale behind the technique is quiet similar to those of any other muscle release intervention. When a trigger point is being palpated by direct finger or thumb pressure, and when the very tissues in which the trigger point lies are positioned in such a way as to take away the pain (entirely or at least to a great extent), then the most stressed fibres in which the trigger point is housed are in a position of relative ease. At this time the trigger point would be under direct inhibitory pressure (mild or perhaps intermittent) and would have been positioned so that the tissues housing it are relaxed (relatively or completely). Following a period in this position of ease and inhibitory pressure, the patient is asked to introduce an isometric contraction into the tissues and to hold this for 5-7 seconds – involving the precise fibres that had been repositioned to obtain the positional release. The effect of this would be to produce (following the contraction) a reduction in tone in these tissues. The hypertonic or fibrotic tissues could then be gently stretched as in any muscle energy procedure so that the specifically targeted fibres would be stretched following an isometric contraction. The technique is proven to be effective in Non- Specific Myofascial Pains, Trigger Point Pain, Muscle Spasms, Fibromyalgia Syndrome and some other myofascial conditions.\textsuperscript{(8)}

As this technique is found to be effective in these conditions, this made a scope for this technique to be experimented in Frozen Shoulder. In order to find out the effectiveness of the current technique in Frozen Shoulder and enable faster and smoother prognosis of subjects with Frozen Shoulder towards normal functioning, the current study was undertaken.

**Materials and Methodology**

**Study Type:** Experimental study.

**Study Design:** Comparative study (Pre treatment and Post Treatment)

**Place of Study:** Karad.

**Sampling Method:**

Simple Random Sampling.

**Sample Size:**

\[ n = 4 \frac{pq}{L^2} \]

Total number of subjects in study \( n = 40 \)

**Inclusion Criteria**

1. Both male and female participants willing to
participate in the study.

2. Age: 40-65 Years

3. Subjects clinically diagnosed with frozen shoulder by certified physiotherapist/Orthopaedician

4. Subjects in second stage of frozen shoulder.

**Exclusion Criteria**

1. Recent Trauma around Shoulder.

2. Recent fracture around the shoulder.

3. Open wound or skin disease.


5. Previous shoulder surgeries.

6. Other shoulder pathologies such as Rotator cuff tear or Chronic Regional Pain Syndrome.

**Outcome Measures**

1. Visual Analogue Scale (VAS). (9)

2. Universal Goniometer. (10)

3. Shoulder Pain And Disability Index (SPADI). (11)

**Procedure**:

The study was carried out and the result was drawn by using VAS, SPADI and Joint ROM scores as the outcome measures. 40 patients (23 Males and 17 Females) were undertaken for the study. The age Group was between 40-65 years. Study place was Krishna College of Physiotherapy, OPD. Patients were evaluated and were divided into 2 Groups by convenient sampling with random allocation. Group A included 20 subjects treated with hot moist pack, Ultrasound, Exercises and Stretching (capsular and sleeper). Group B included 20 subjects treated with hot moist pack, Ultrasound, Exercises and Stretching (capsular and sleeper) and Integrated Neuromuscular Inhibition Technique. An informed Consent was taken from the subjects once they were filtered by the inclusion and exclusion criteria. Subjects were asked to fill the data collection sheets and were treated for 7 days after which. The scores of the outcome measures were measured post-procedures.

**Findings**

Statistical analysis was done manually and by using the statistics software INSTAT so as to verify the results derived. The statistical analysis within group value of VAS, ROM and SPADI was done by Paired ‘t’ test. Unpaired ‘t’ test was used for interpretation of post interventional values between Group A and Group B. Fisher’s Exact test was used for calculating the difference in the age groups and occupation.

**Table 1: Gender Distribution.**

A total of 40 subjects were taken for the study. Out of 40 subjects 23 were males and 17 were females.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Group A</th>
<th>Group B</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>12</td>
<td>11</td>
<td>23</td>
</tr>
<tr>
<td>Females</td>
<td>8</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>20</strong></td>
<td><strong>20</strong></td>
<td><strong>40</strong></td>
</tr>
</tbody>
</table>

**v Table 2: Age Distribution.**

Age group of all patients ranged between 40-65 years with the mean age of Group A was 49 and Group B was 49.85 years.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean Age (Yrs) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>49 ± 6.456</td>
</tr>
<tr>
<td>Group B</td>
<td>49.85 ± 6.515</td>
</tr>
</tbody>
</table>

Intra group analysis of all pre and post interventional values was done by Paired ‘t’ test. Inter group analysis of all values was done by using Unpaired ‘t’ test. Post intervention analysis of VAS showed extremely significant difference between both groups (p=0.002). Post intervention analysis showed very significant difference between both groups for SPADI (p=0.0047), FLEXION ROM (p=0.0076) and ABDUCTION ROM (p=0.0026). There was no significant difference between the two groups regarding MEDIAL ROTATION ROM (p=0.1772) and LATERAL ROTATION ROM (p=0.7602).
Table 3: Comparison of baseline parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group A</th>
<th>Group B</th>
<th>p value</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAS</td>
<td>3.75 ± 0.9665</td>
<td>2.4 ± 1.095</td>
<td>0.002</td>
<td>Extremely significant</td>
</tr>
<tr>
<td>SPADI</td>
<td>68.1 ± 14.878</td>
<td>52.75 ± 17.363</td>
<td>0.0047</td>
<td>Very significant</td>
</tr>
<tr>
<td>FLEXION ROM</td>
<td>125 ± 15.570</td>
<td>137.45 ± 12.150</td>
<td>0.0076</td>
<td>Very significant</td>
</tr>
<tr>
<td>ABDUCTION ROM</td>
<td>123.55 ± 14.947</td>
<td>136.35 ± 9.522</td>
<td>0.0026</td>
<td>Very significant</td>
</tr>
<tr>
<td>MEDIAL ROTATION ROM</td>
<td>44.25 ± 7.684</td>
<td>47.45 ± 7.022</td>
<td>0.1772</td>
<td>Not significant</td>
</tr>
<tr>
<td>LATERAL ROTATION ROM</td>
<td>41.1 ± 7.622</td>
<td>41.8 ± 6.756</td>
<td>0.7602</td>
<td>Not significant</td>
</tr>
</tbody>
</table>

Discussion

A total of 23 males and 17 females were divided in two groups randomly. Group A consisted of 12 males and 8 females, whereas Group B consisted of 11 males and 9 females. The difference between the two groups regarding Gender was found to be not significant. Although Frozen Shoulder is found to be more common in females, the current study had more number of males than females. The possible reasons behind this might be a small sample size and the characteristic flow of patients in the locality.

The mean age of subjects in Group A was 49 ± 6.456 and in Group B was 49.85 ± 6.515. With a p value of 0.5010 the difference in mean age of the two groups was found to be statistically not significant.

In Group A, which is the controlled group, extremely significant difference was obtained by the use of Hot moist pack, Therapeutic Ultrasound, Codman’s Pendular Exercises, Capsular Stretching and Sleeper Stretch. The rationale behind each of the treatment and their reasonable effect can be stated as follows. Hot moist packs that transfer heat from the packs to the patient’s body increase the temperature of the respective body part. With increase in temperature, the stress-relaxation ability of the collagen fibres is enhanced, which enable larger deformation in these fibres when subjected to stretch.

Therapeutic Ultrasound on the other hand has been proven in previous studies also for elevating collagen tissue extensibility, pain threshold and enzymatic reactions. Also, it causes alteration in nerve conduction velocity, and the contractility of skeletal muscles.

Codman’s Pendular exercises are a form of exercises that utilize the effects of gravity to provide a distraction of the humeral head from the glenoid fossa. These exercises help reduce pain via gentle traction and revolving movement and enhance early motion of the articulating parts and the joint synovial fluid.

Capsular Stretching and Sleeper Stretch follow the effects of general stretching with added benefit of going through the capsular pattern of the joint and increasing soft tissue mobility and further increasing joint Range of Motion by elongating the shortened and hypo mobile structures.

In Group B, which is the experimental group, extremely significant difference was obtained by
combining the above-mentioned conventional treatment with a new technique called Integrated Neuromuscular Inhibition Technique. This group showed significant difference when compared with the control group post-interventionally.

The added benefit obtained in this group goes to explain the rationale of use of a Manual Muscle releasing technique which was not used in the control group.

Muscle release interventions have been proven to improve blood circulation, reduce pain, alter the excitability of the alpha and delta motoneurons and thus enhance joint range of motion.\(^{(16)}\)

Studies prove that the Ischemic Compression component in the INIT decreases the threshold sensitivity of the pain nodules in the muscles. It is proposed that local pressure tends to equalize the length of sarcomeres in the involved TrP and thus reduces pain.\(^{(17)}\) Additionally, a mechanism called ‘unopposed arterial filling’ has also been put forward which enables improvement in tone of the muscles.\(^{(18)}\)

The P value for MEDIAL ROTATION ROM \((0.1772)\) and LATERAL ROTATION ROM \((0.7602)\) showed that the rotational movements ROM in the two groups did not differ significantly. This may have happened because these two movements being performed on a different axis and with shorter moment arms did not require much of muscle point pain corrections.

In this study an attempt was made to analyze the effect of Conventional Treatment and Integrated Neuromuscular Inhibition Technique in relieving pain and improving Joint Range of Motion as well as functional status and strength in Frozen Shoulder patients. This study was done to investigate the reduction of symptoms after application of Integrated Neuromuscular Inhibition Technique in conjunction with Conventional Treatment in Frozen Shoulder patients and its post treatment evaluation in a standardized manner using VAS, SPADI and ROM scores. The result shows that there is significant difference in improvement of pain, functional performance, Flexion and Abduction Range of Motions between the two groups. The group treated with Integrated Neuromuscular Inhibition Technique showed significantly better results for these outcome measures. Although, there was no significant difference regarding Medial and Lateral Rotation Range of Motions between the two groups.

**Conclusion**

The current study concluded that Integrated Neuromuscular Inhibition Technique and Conventional Physiotherapy are both effective in the management of Frozen Shoulder. Also, Integrated Neuromuscular Inhibition Technique given with Conventional Treatment is significantly more effective as compared to conventional treatment alone on pain, range of motion and functional status of Shoulder and thus Alternate Hypothesis accepted.

**Conflict of Interest:** There is no conflict of interest concerning the content of the study.

**Source of Funding:** This study was funded by Krishna institute of medical sciences deemed university, karad

**Ethical Clearance:** The study was approved by the institutional ethics committee of KIMSDU.

**References**


Comparison of Closed Kinetic Chain Exercises and Conventional Therapy on Cardiac Parameters in Post CABG Subjects

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Abstract

Introduction: Cardiovascular disorders are public health problems worldwide. In industrialized world they are the leading cause of morbidity and mortality. Coronary artery bypass grafting (CABG) has proved to be a major life saving operative method in cardiovascular disease. Physiotherapy interventions play major role in rehabilitation post CABG so the study was conducted with the aim to find the effect of closed kinetic chain exercises and conventional therapy on cardiac parameters in post CABG subjects.

Method: In this comparative study 20 subjects were treated for post CABG subjects between the age group of 40-50 years. Group A were given conventional treatment and Group B were given Closed Kinetic Chain exercise. The pre and post -test was measure blood pressure, respiratory rate, heart rate, oxygen saturation and borg scale.

Results: The statistical analysis for conventional therapy pre and post week1 day 1-systolic blood pressure(p=<0.0001), diastolic blood pressure(p=0.005), respiratory rate(p=0.001), heart rate(p=0.011), oxygen saturation(p=0.081) and borg scale(p=0.003). pre and post week6 day 3- diastolic blood pressure(p=0.018), respiratory rate(p=<0.0001), heart rate(p=0.005), systolic blood pressure(p=0.186), oxygen saturation(p=0.343) and borg scale(p=0). The statistical analysis for experimental group pre and post week1 day 1-heart rate (p=0.01) diastolic blood pressure(p=0.002), systolic blood pressure(p=<0.0001), respiratory rate(p=<0.0001), borg scale(p=0.0007), oxygen saturation (p=0.508). pre and post week6 day 3-borg scale(p=0), diastolic blood pressure(p=0.051), heart rate (p=<0.0001), respiratory rate(p=<0.0001), systolic blood pressure(p=0.26) and oxygen saturation(p=0.34).The study had found that conventional therapy is more effective than experimental group.

Conclusion: The study concluded that conventional treatment is more effective than closed kinetic chain exercises. However, it cannot be stated that CKC has no effect because of its additional benefits on functional capacity (improve joint integrity). Therefore, it can be concluded that further studies can evaluate the improvement noted with a right combination of both and obtain greater benefits.

Keyword: post CABG, cardiac parameters, closed kinetic chain, oxygen saturation, blood pressure

Introduction

Cardiovascular disorders are public health problems worldwide. In industrialized world they are the leading cause of morbidity and mortality. The annually recorded cardiovascular mortality is about 0.8%.1 In India cardiovascular diseases account for 25 % of death rate.2

Coronary artery bypass grafting(CABG) has proved to be a major life saving operative method and thereby aiding in prolongation of life, primarily by help relieving the symptoms of angina.3

The major complication that emerge after surgery are being bed ridden, oedema, wound pain, decreased physical activities and depression.4 In previous study
by Jenkins et al 1983, breathlessness, or dyspnea were investigated. It was found that 60% of patients experienced dyspnea before CABG, 54% of these were completely relieved of dyspnea, 22% reported some improvement, and 18% had no improvement 6 months following surgery. Another similar study by Mayou and Bryant 1987 reported that 71% of patients experienced dyspnea before surgery whilst 39% reported it 12 months post-surgery. In a study by Duprez D. it was found that during rehabilitation phase mean ambulatory blood pressure parameters were within normotensive range. Studies state Surgery affects physical activity, endurance, muscle strength, work in performance and quality of life to a certain extent.

In a study ShifaManhal found that the range of motion and function of the shoulder girdle and upper back are impaired following CABG, specially after median sternotomy, due to retraction of sternum. The chances of developing frozen shoulder increases after cardiovascular surgery. Thus broad spectrum of musculoskeletal problems affects quality of life of cardiac patients.

Cardiac rehabilitation (CR) is an outpatient secondary prevention program comprised of structured exercise training as well as comprehensive education and counselling. Therefore, it is necessary and considered useful for the patients. Numerous studies suggest greater physical fitness and quality of life after receiving exercise training for a long time and post-surgery education. Various physiotherapy approaches till date are Active exercises for upper and lower limb Breathing exercises, Inspiratory diaphragmatic breathing exercise, Expiratory pursed lip breathing exercise, walking, stair climbing, spirometry primarily addressing for return of quality of life post CABG.

In a study it was found that mean grade scores of physical limitations were significant pre-treatment with a substantial improvement post treatment. Exercise based rehabilitation lowers rate of cardiac death. Findings from a review of 22 randomized control trails revealed a 20% reduction in mortality rate.

Despite of these many musculoskeletal complications there are very few and limited studies performed and there are many reasons to believe the occurrence of shoulder dysfunction with increasing population of postoperative CABG patients.

Till date many approaches are carried out to overcome physical limitation primarily OKC approach. But no studies till date report the significance of CKC exercise alone. Therefore it is need to study the effect of Closed Kinetic Chain (CKC) exercises as these exercises have maximal surface contact and distal segment is fixed and movement of proximal joint. There is activation of many group of muscle while movement of joint takes place which might add to a better improvement in reducing physical limitations. However only caution regarding the proportion of drop outs need to be interpreted. However, a program of exercise that does not include all exercise components or achieve less than the recommended volumes of exercises is likely to have benefit, particularly in habitually inactivate persons.

**Material and Methodology**

An approval for the study was obtained from the Protocol committee and institutional Ethical Committee of KIMSDTU. 20 subjects were included post 6 week of CABG and between age group of 40 to 50 and those who have undergone CABG for the first time. Subjects with Neurological problems, musculoskeletal problems before surgery, with movement disability and impaired cognition were excluded. Subjects were explained about the procedure of the study and written consent was taken. Pre and Post assessment (week 1 day 1) of Blood pressure, Heart rate, Respiratory rate, oxygen saturation was taken to assess the subject. Target heart rate was decided according to age-adjusted predicted maximum heart rate formula: 220 – age. A total 20 subjects was equally divided into two groups. Group A (conventional) received active exercises for upper and lower limb, Breathing exercises, walking, stair climbing and spirometry where Group B (experimental) received standing wall side, partial squat, lunges, wall push up and dorsiflexion and push the wall.
Findings

1. Comparison of Pre and Post within Group A

Table no 1: baseline parameters in group A

<table>
<thead>
<tr>
<th>Parameters</th>
<th>w1 d1</th>
<th>Post</th>
<th>w6 d3</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP st</td>
<td>140.6±3.56</td>
<td>144.1±3.63</td>
<td>129.6±6.72</td>
<td>131.0±6</td>
</tr>
<tr>
<td>BP dy</td>
<td>84.900±4.508</td>
<td>88.800±3.676</td>
<td>80±6.667</td>
<td>84.1±8.062</td>
</tr>
<tr>
<td>RR</td>
<td>29.2±3.565</td>
<td>31.6±4.115</td>
<td>21.4±1.35</td>
<td>22.2±1.033</td>
</tr>
<tr>
<td>HR</td>
<td>91.1±5.705</td>
<td>101.1±12.512</td>
<td>73.6±2.27</td>
<td>83.6±10.23</td>
</tr>
<tr>
<td>OS</td>
<td>96.3±1.889</td>
<td>96.6±1.578</td>
<td>99.3±0.948</td>
<td>99.5±0.849</td>
</tr>
<tr>
<td>BS</td>
<td>0.05±0.1158</td>
<td>0.45±0.3689</td>
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<table>
<thead>
<tr>
<th>Parameters</th>
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</thead>
<tbody>
<tr>
<td>BP st</td>
<td>-3.5</td>
<td>10.24</td>
<td>&lt;0.001</td>
<td>S</td>
</tr>
<tr>
<td>BP dy</td>
<td>-3.9</td>
<td>3.578</td>
<td>0.0059</td>
<td>S</td>
</tr>
<tr>
<td>RR</td>
<td>-2.2</td>
<td>7.571</td>
<td>&lt;0.001</td>
<td>S</td>
</tr>
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<td>NQS</td>
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<td>0.0031</td>
<td>VS</td>
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2. COMPARISION OF PRE AND POST WITHIN GROUP B

Table no 2: baseline parameters in group B

<table>
<thead>
<tr>
<th>Parameters</th>
<th>w1 d1</th>
<th>Post</th>
<th>w6 d3</th>
<th>Post</th>
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<tbody>
<tr>
<td>BP st</td>
<td>136±5.16</td>
<td>141.7±6.23</td>
<td>132±3.49</td>
<td>134±6.58</td>
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<tr>
<td>BP dy</td>
<td>86±9.36</td>
<td>89.2±8.92</td>
<td>82±7.52</td>
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</tr>
<tr>
<td>RR</td>
<td>31.2±2.098</td>
<td>37±2.906</td>
<td>20.8±2.34</td>
<td>24.7±2.98</td>
</tr>
<tr>
<td>HR</td>
<td>79.4±9.74</td>
<td>96.9±9.89</td>
<td>71.8±1.68</td>
<td>76.8±2.48</td>
</tr>
<tr>
<td>OS</td>
<td>96.6±1.83</td>
<td>96.8±1.317</td>
<td>99.8±0.63</td>
<td>100±0</td>
</tr>
<tr>
<td>BS</td>
<td>0.05±0.158</td>
<td>1.25±0.716</td>
<td>0</td>
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</table>
3. **COMPARISION OF PRE AND POST BETWEEN GROUP A AND GROUP B**

Table no 3: baseline parameters in group A and B

<table>
<thead>
<tr>
<th>Parameters</th>
<th>grp A &amp; B</th>
<th>BP st</th>
<th>BP dy</th>
<th>RR</th>
<th>HR</th>
<th>OS</th>
<th>BS</th>
</tr>
</thead>
<tbody>
<tr>
<td>w1 d1 post</td>
<td>A</td>
<td>144.1±3.63</td>
<td>88.800±3.676</td>
<td>31.600±4.115</td>
<td>101.10±12.512</td>
<td>96.60±1.578</td>
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</tr>
<tr>
<td>w6 d3 post</td>
<td>B</td>
<td>141.7±6.23</td>
<td>89.200±8.929</td>
<td>37.00±2.906</td>
<td>96.90±9.893</td>
<td>99.800±0.3162</td>
<td>1.25±0.716</td>
</tr>
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</table>

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<td>141.7±6.23</td>
<td>131.00±6.000</td>
<td>134.00±6.583</td>
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<td>w6 d3</td>
<td>88.800±3.676</td>
<td>89.200±8.929</td>
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<td>23.200±1.033</td>
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<td>BP dy</td>
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<td>RR</td>
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<td>BS</td>
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**Discussion**

This present study “comparison of closed kinetic chain exercises and conventional therapy on cardiac parameters in post CABG subjects” was conducted to interpret impact CKC exercises on cardiac parameters such as blood pressure, respiratory rate, heart rate, oxygen saturation, borg scale. Post CABG decreased physical functioning has been noted. Recent study states that an investigation of exercise behaviour at 6 and 24 months post-surgery 67% of individuals were regular exercisers and therefore retaining pre-activity level and reducing deterioration of physical activity following surgery.5 CKC exercises improve joint integrity of the individuals, as these exercises have maximal surface contact and distal segment remained fixed.

The outcome measures used in study were Blood pressure, Heart rate, Respiratory rate, oxygen saturation
and Target heart rate.

Group A: pre and post week1 day 1-There was significant changes in systolic blood pressure (p=<0.0001), diastolic blood pressure (p=0.005), respiratory rate (p=0.001), heart rate (p=0.011), not quite significant change in oxygen saturation (p=0.081) and very significant change in borg scale (p=0.003). pre and post week6 day 3- there was significant changes in diastolic blood pressure (p=0.018), respiratory rate (p=<0.0001), very significant changes in heart rate (p=0.005), not significant change is systolic blood pressure (p=0.186), oxygen saturation (p=0.343) and borg scale (p=0).

Group B: pre and post week1 day 1-There was significant changes in heart rate (p=0.01) very significant change in diastolic blood pressure (p=0.002), extremely significant changes in systolic blood pressure (p=<0.0001), respiratory rate (p=<0.0001), borg scale (p=0.0007), not significant changes in oxygen saturation (p=0.508), pre and post week6 day 3- there was significant changes in borg scale (p=0), not quite significant change in diastolic blood pressure (p=0.051), extremely significant heart rate (p=<0.0001), respiratory rate (p=<0.0001) not significant change in systolic blood pressure (p=0.26) and oxygen saturation (p=0.34).

Between group comparison: Group A and B: post week1 day 1 – significant change in heart rate (p=0.415), very significant change in respiratory rate (p=0.003) and borg scale (p=0.005), not significant change in systolic blood pressure (p=0.307), diastolic blood pressure (p=0.897) and oxygen saturation (p=0.761) Group A and B: post week6 day 3- significant change in borg scale (p=0), not quite significant change in heart rate (p=0.056), not significant change in systolic blood pressure (p=0.30), diastolic blood pressure (p=0.77), respiratory rate (p=0.15) and oxygen saturation (p=0.18). Also a study by Gabriela Lima de Melo Ghissiit was found that cardiologist lack knowledge regarding CR and therefore barrier to secondary prevention. The major limitation of this study was the unwillingness of the patients to participate in the study. This study addresses the gap of adding CKC components in conventional treatment although with caution. Further studies need to be done on different age groups, different geographical area, for a longer duration, adding different stages of rehabilitation phase.

**Conclusion**

The study concluded that conventional treatment is more effective than closed kinetic chain exercises. However, it cannot be stated that CKC has no effect because of its additional benefits on functional capacity (improve joint integrity). Therefore, it can be concluded that further studies can evaluate the improvement noted with a right combination of both and obtain greater benefits.

**Conflict of Interest:** None

**Source of Funding:** This work was supported by the funding of KRISHNA INSTITUTE OF MEDICAL SCIENCES “DEEMED TO BE UNIVERSITY” Karad, Maharashtra.

**Ethical Clearance:** The study has been ethically cleared by institutional ethical committe of KIMSDU, Karad

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Effect of Step Aerobics Applied Early in Phase 2 Cabg Subjects on Functional Capacity and Exercise Tolerance

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¹Internee, Faculty of Physiotherapy, ²Associate Professor, Department of Cardio-Pulmonary sciences, Krishna Institute of Medical Sciences ‘Deemed to be’ University, Karad, Maharashtra, India

Abstract

Background: In recent years, cardiovascular accidents are rising due to improper diet, ill habits, uncontrolled cholesterol levels, stress, etc. Coronary artery diseases are in the leading cause of death. After a coronary artery disease like myocardial infarction the individual’s general health is week and the quality of life of these individuals is reduced and hence, morbidity starts building, causing reduced functioning and reduction in overall health and functional capacity. 30 individuals participated in this study who had undergone coronary artery bypass grafting and were under phase 2 of cardiac rehabilitation. The individuals had performed low intensity Step Aerobics for 12 weeks and were evaluated for the efficiency of step aerobics on functional capacity and exercise tolerance in such individuals.

Objectives: To find the effect of step aerobics on the functional capacity and on exercise tolerance.

Methodology: In this study, 30 subjects who had undergone CABG of age from 30-65 years. These individuals were selected on the basis of their inclusion criteria. They were treated with step aerobics as form of exercises for 12 weeks. After pre-post assessment, the data was analysed with help of proper statistical methods.

Results: According to the result, there is extremely significant difference among the subjects six-minute walk test distance scores (p-value < 0.0001). Among the vitals assessed pre and post six-minute walk test, there was significant difference between the respiratory rate (p-value=0.0219), there was also very significant difference between the heart rate (p-value < 0.001). There was not quite significant change in the blood pressure levels i.e. systolic and diastolic p-value=0.0559 and 0.0862 respectively. Also, there was seen extremely significant difference in the peak O₂ consumption levels according to DASI p-value < 0.0001) and also in the MET scores in DASI (p-value < 0.0001), making the difference to be extremely significant.

Conclusion: The results obtained by studying the effects post treatment suggest that in rehabilitation of the CABG patients, implementation of step aerobics early has greater effects on functional capacity, exercise tolerance and general health including the quality of life.

Keywords: Coronary artery bypass grafting (CABG), step aerobics, functional capacity, exercise tolerance, peak oxygen consumption, Duke Activity Status Index (DASI).

Introduction

Coronary artery bypass grafting (CABG) is the most common open-heart surgery for myocardial infarction patients with major coronary artery blockages due to plaques. According to the Acute Coronary Treatment and Intervention Outcomes Network Registry in 2012, the NSTEMI subjects who did not have a past surgical history of CABG and subjects with three-vessel or left main coronary disease during angiography approximately 40% underwent CABG¹¹. Coronary artery disease is the leading cause of death worldwide. 3.8 million men and 3.4 million women die each year of coronary artery
diseases on global scale. Over last 60 years, prevalence has increased from 1% to 9-10% in urban areas and from <1% to 4-6% in rural areas. According to the survey of 2016, it was reported that 28.1% of total deaths due to coronary artery diseases. The prevalence of coronary artery disease in India was estimated about 23.8 million cases in 2016.

This surgery has complications such as postoperative pulmonary complications, changes in lung mechanics, restrictive breathing pattern and shallow breathing. Atelectasis is common in these cases causing reduced lung capacity and respiratory muscle strength. Due to sternotomy among these patients, immobility in bed and temporary dysfunction in diaphragm causes hypoxia and postoperative pulmonary dysfunction. Ejection fraction is reduced in these patients due to left ventricular ischemia. These all factors cause reduced functional capacity further poor quality of life.

CABG causes reduced peak oxygen consumption ($VO_{2max}$). Due to reduced aerobic capacity, reduction in the functional capacity of these individuals is also reduced due to morbid state and less functioning. They have shown great effects on improving the aerobic capacity and functional capacity. They improve cardiac output, $VO_{2max}$ and enhance the ability of the muscles to utilize the oxygen from blood. Studies have shown aerobic exercises to improve peak $VO_2$ up to 46%. Systolic function also increased by 35%, hence strengthening the advantages of aerobic exercises.

Physical activity is needed in these subjects along with fair interaction with the family and friends. Also, physical training causes decrease in body weight and improve emotional health and quality of life. Vascularity of muscles increases and peripheral oxygenation improves due to physical activity. Physical activity guidelines recommend that every healthy adult should engage in 92 minutes or more of low to moderate intensity physical exercises for a week.

Aerobic exercises have great effects on the functional capacity and exercise tolerance. Aerobics has all the benefits of a general high intensity workout. Aerobic exercise is believed to play a part in lowering the risk of cardiovascular disease by increasing the serum levels of high-density lipoprotein cholesterol. Effects of aerobic exercises on depression and anxiety are also seen. Mainly, it plays an important role in controlling the blood pressure and the blood sugar levels, reducing the risk of coronary artery diseases. In CABG subjects, aerobic exercises play an important role in most common contributory factor for morbidity, viz., peak oxygen uptake ($VO_{2max}$).

Studies have shown that aerobic exercises have great effects on the cardiovascular and physical functioning. Step aerobics also has shown great effects on cardiopulmonary functions and exercise tolerance. Previous studies have shown effects on cardiopulmonary fitness and cholesterol levels in young women with sedentary lifestyle, also some studies have shown the energy expenditure due to walking along with stair climbing and its effect on physical fitness.

Step aerobics is an up-tempo way to get your heart pumping and stay fit. Step aerobics training involves stepping up and down on a single bench/step in choreographed, group-led movements to music. Step aerobics intensity can vary from low to moderate intensity in health compromised subjects and moderate to high intensity in obese to normal subjects. It is a form of exercise to get benefits like high intensity cardio workout without putting stress on your joints. This can be done in presence or absence of music. It not only acts as a cardio workout but also has an effect on strength of legs.

Addition of low intensity exercises in phase 2, may induce increase in functional capacity and exercise tolerance. Hence, step aerobics should be incorporated in daily functional training for greater results.

**Methodology**

The ethical clearance was taken from ethical committee of Krishna institute of Medical Sciences, Karad. There were 30 participants in the study. The study was taken place in the Krishna Hospital. Treatment protocol was of 12 weeks. The subjects were assessed for functional capacity using six-minute walk test at first and exercise tolerance using the Duke Activity Status Index. The type of study was experimental study. The study design was pre-post.

**Procedure**

All the subjects for the study were selected according to the selection criteria. Demographic data and written consent were obtained from the subjects. The subjects...
were explained about the protocol and demonstrated. Pre and post assessment were taken before and after 12 weeks of the treatment period respectively with the help of outcome measures. Pre-treatment exercise tolerance was assessed using 6-minute walk test. Pre-vitals, blood pressure, heart rate, respiratory rate was assessed before starting the test. The patient was asked to walk a distance of 30m to and fro for 6 min at normal pace. There were chairs kept at regular interval for the patient to rest if fatigue or breathless. After completion or before completion of 6 minutes i.e. when the patient was found to be restless while walking, the patient was again assessed for vitals and noted. The distance covered by the patient in 6 minutes was calculated and kept as pre-treatment record. The patients were also given Duke Activity Status Index for functional assessment and rough estimation of the oxygen uptake and exercise tolerance. The patients according to the exercise tolerance calculated were trained with step aerobics for 12 weeks. Every session lasted for 45 minutes of step aerobics including the warm up and cool down exercises. The warm up lasted for 5 minutes which included neck flexion-extension, rotation, arm rotations, trunk lateral flexion, trunk rotation and basic stretches arm and legs. Once done with warm up, the protocol was performed in the sequence of basic right, basic left, A-step move, across the top move with deep breathing. Each move lasted for 1 minute and repeated after every 5 minutes. The exercises continued for 25 minutes and then followed by cooling down of the body which consisted of arm shaking, legs shaking, deep breathing. After completion of 12 weeks, the subjects had to undergo 6-minute walk test and DASI for the exercise tolerance and functional capacity assessment respectively. The data was collected and recorded. Later statistical analysis was performed.

Results

1. Distribution according to demographic data

Table no. 1: Distribution according to demographic data in study.

<table>
<thead>
<tr>
<th>AGE</th>
<th>Participants</th>
<th>Percentage</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>100%</td>
<td>53.57±6.52</td>
<td></td>
</tr>
</tbody>
</table>

| GENDER | MALE | 56.67% | - |
|        | FEMALE | 43.33% | - |

| BMI     | NORMAL | 43.33% | 23.24±0.81 |
|         | OVERWEIGHT | 50.0% | 27.56±1.58 |
|         | G-1 OBESE | 6.66% | 32.55±1.77 |

Interpretation: The above table depicts a picture of demographic data distribution with components as Age, Gender and Body-Mass Index (BMI). Out of 30 subjects, 56.67% were male and 50% were overweight according to calculated BMI.

2. Distribution and association of scores of 6-min walk test

Table no. 2: Distribution and association of 6-min walk test scores

<table>
<thead>
<tr>
<th>6-MIN WALK TEST</th>
<th>Mean±SD</th>
<th>Paired t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre intervention</td>
<td>197.67±21.76</td>
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</tr>
<tr>
<td>Post intervention</td>
<td>286.33±37.36</td>
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</tbody>
</table>
Interpretation: Above table represents mean score of 6-minute walk test distance scores pre and post intervention.

3. Distribution and association of Vital scores in Six-minute walk test

Table no. 3: Distribution and association of vital sign scores

<table>
<thead>
<tr>
<th>Vitals</th>
<th>Mean±SD</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre intervention</td>
<td>Post intervention</td>
</tr>
<tr>
<td>Blood Pressure</td>
<td>Systolic</td>
<td>Diastolic</td>
</tr>
<tr>
<td></td>
<td>132.23±1.36</td>
<td>94.41±2.48</td>
</tr>
<tr>
<td></td>
<td>Pre intervention</td>
<td>Post intervention</td>
</tr>
<tr>
<td>Heart Rate</td>
<td>106.14±4.65</td>
<td>88.32±1.41</td>
</tr>
<tr>
<td>Respiratory Rate</td>
<td>22.86±2.13</td>
<td>19.34±0.18</td>
</tr>
</tbody>
</table>

Interpretation: Above table represents mean score of vitals i.e. blood pressure, heart rate and respiratory rate and its association pre and post treatment.

4. Distribution and association of peak O2 scores of Duke Activity Status Index (DASI)

Table no. 4: Distribution and association of DASI scores for peak O2

<table>
<thead>
<tr>
<th>Duke Activity Status Index (DASI)</th>
<th>Mean±SD</th>
<th>Paired t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre intervention</td>
<td>Post intervention</td>
</tr>
<tr>
<td></td>
<td>16.81±2.51</td>
<td>20.42±2.41</td>
</tr>
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</table>

Interpretation: Above table represents the mean score of peak oxygen consumption levels pre and post intervention calculated using DASI.

5. Distribution and association of MET scores of Duke Activity Status Index (DASI)

Table no. 5: Distribution and association of DASI scores for MET

<table>
<thead>
<tr>
<th>Duke Activity Status Index (DASI)</th>
<th>Mean±SD</th>
<th>Paired t-test</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Pre intervention</td>
<td>Post intervention</td>
</tr>
<tr>
<td></td>
<td>4.80±0.71</td>
<td>5.83±0.69</td>
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</table>
Interpretation: Above table represents the mean score of metabolic equivalents (MET) pre and post intervention calculated using DASI.

Discussion

Aerobic exercises are the type of exercises which have effects of the aerobic capacity and improve the oxygen supply and avoid early fatigue. Step aerobic exercises not only improve the aerobic capacity but also helps in building the balance and agility in middle aged and old age population. It mainly has effects on the body composition in young and adult population. The repetitive stepping up and down improves the lower and upper body strength In this study, 30 individuals were selected on the basis of the inclusion and exclusion criteria. The mean age of these participants was 53.57 years. Out of the total participants, 13 (43.33%) were female and 17 (56.67%) were male.

6-min walk test was used to assess the participant’s functional capacity. It was performed at the commencement of the treatment i.e. in the beginning of 1st week and at the completion of treatment i.e. at the end of the 8th week. The mean score of participant’s distances covered pre intervention was 197.66 metres and the mean score of participant’s distance post intervention was 286.33 metres. Thus, there was a considerable increase in the distance traversed by the participants post administration of treatment.

The vitals of the participants were also assessed during the 6-minute walk test. Blood pressure (BP), heart rate and respiratory rate were the vitals assessed. The mean score of systolic BP pre intervention was 132.23 mmHg and mean systolic BP score post intervention was 125.05 mmHg. Similarly, mean diastolic BP score pre and post intervention were 94.41 mmHg and 87.17 mmHg respectively. The mean score of heart rate were also calculated similarly, mean score of heart rate pre intervention was 106.14 beats/min and that post intervention was 88.32 beats/min. Respiratory rate mean scores were also calculated in the same way, the pre intervention mean score was 22.86 breaths/min and the post intervention mean score was 19.34 breaths/min.

Duke Activity Status Index (DASI) was used to assess the participant’s estimate peak oxygen consumption and their exercise capacity on the basis of Metabolic Equivalent of Task (MET).

The questionnaire was provided to the participants before the commencement and after completion of the treatment protocol. The scores were then calculated. The mean score for peak oxygen consumption pre intervention 16.81 was and the mean score post intervention was 20.42. This shows that there was an increase in the peak oxygen consumption levels of the participants in turn improving their functional capacity.

The mean score pre intervention of MET was 4.80 and the mean score post intervention for MET was 5.83. Therefore, there is considerable improvement in the ability of the participants capacity to perform tasks as a result of the increased exercise tolerance.

Intra-group association for score of 6-min walk test within the population; pre and post intervention was done. Paired t-test was used which had a p-value of <0.0001 which was extremely significant with a t-value of 15.757. Thus, there is a significant increase in the distance covered by the participants post administration of our exercise protocol. Similarly, paired t-test was used to calculate the association between the vitals i.e. systolic BP which had a p-value of 0.559 which was not quite significant with a t-value of 2.019, diastolic BP with a p-value of 0.0862 which was not quite significant with a t-value of 1.796, heart rate with a p-value of <0.001 which was very significant with a t-value of 7.139 and lastly the respiratory rate which had a p-value of 0.0219 which was significant with a t-value of 2.377.

Association of DASI scores for peak oxygen consumption was also analysed, which had a p-value of <0.0001 which was extremely significant with a t-value of 10.866. DASI scores for MET also had a p-value of <0.0001 which is extremely significant with a t-value of 10.856.

In this study, we can say that there is a significant improvement in all the parameters, general health and vitals of the participants. This results in an overall increase in the functional capacity, quality of life and exercise tolerance of the participants. Hence, implementation of our treatment protocol may help individuals undergoing CABG recover faster and turn out healthier than the average patient. It may also improve the confidence in physiotherapists in turn promoting our profession as well improve the patient’s confidence in himself leading to a speedy recovery.
Conclusion

On the basis of the result of the study it is concluded that in rehabilitation of CABG subjects, early implementation of step aerobics has a number of effects on the functional capacity, exercise tolerance and quality of life.

Source of Funding:

Krishna Institute of Medical Sciences Deemed to be University, Karad.

Conflict of Interest: There was no conflict of interest in my study.

Ethical Clearance: The Institutional Ethical Committee has hereby given permission to initiate the research project titled, “Effect of step aerobics applied early in phase 2 CABG subjects on functional capacity and exercise tolerance”

References

Improving the Motility of a Scleral Shell prosthesis with an Acrylic Orbital Implant: A case report

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1Post Graduate Student, 2Associate Professor, 3Professor and Head, 4Professor, Department of Prosthodontics, Manipal College of Dental Sciences, Mangalore, Manipal Academy of Higher Education, Manipal, Karnataka, India

Abstract

Evisceration or enucleation of the eye may be performed due to ocular injury, cosmetic deformity, infection or malignancy. After the surgical management, orbital implants can be placed to improve the cosmetic appearance, improve the motility of the scleral shell prosthesis and restore the loss of orbital volume. Various type of orbital implants are available amongst which, the acrylic orbital implant has gained popularity, over the years, due to its limited complications.

The following case report represents a 26 year old patient who underwent enucleation and had an existing scleral shell prosthesis. His chief concern was the motility of the prosthesis. The prosthetic rehabilitation was done with the help of an Acrylic Orbital implant and a customized scleral shell prosthesis.

Keywords: Eye, Orbital, Implant, Prosthesis, Rehabilitation

Introduction

Eye is a vital organ not only for function but also from an aesthetic point of view. Defects of eye can be classified as ocular or orbital defects which can be due to enucleation, evisceration and exenteration. These defects have aesthetic, psychological and physiological impact on the patient. Prosthetic rehabilitation of these defect often includes ocular and orbital prostheses.1 Acrylic ocular prosthesis can be stock or custom-made. Stock prosthesis are used for interim and post-operative purposes. Custom ocular prosthesis involves impression making of the affected socket and subsequently molding the scleral blank to achieve excellent adaptation with tissues.2 Custom ocular prosthesis has several advantages which include better eyelid movements, reduced incidence of ulceration, improved fit, better comfort, improved facial contours, and enhanced aesthetics gained from the control over the size of the iris, pupil and colour of the iris and sclera.3

Case Report

A 26-year-old male patient reported to the Department of Prosthodontics with the chief complaint of a non-motile artificial eye in his right socket (Figure 1). He lost his right eye in a road traffic accident a year ago and has been wearing the scleral shell prosthesis ever since. Examination revealed enucleation of the right eye with a healthy socket mucosa. Evaluation of the socket depth was deemed sufficient to retain an acrylic orbital implant followed by a scleral shell prosthesis for optimal fit and aesthetics. The Ophthalmologist then carried out the peritomy surgery by opening the conjunctival sac and tenon’s capsule under local anaesthesia and placement of the 18 mm acrylic implant(Figure 2). The conjunctival sac was then closed with 5-0 vicryl sutures. The eye was then patched up and post operative antibiotic coverage was prescribed.

The patient was then recalled after an 8 week period for the fabrication of the ocular prosthesis.
On examination after 8 weeks, the post operative healing was deemed satisfactory and there were no signs of implant extrusion. The patient was then prepped for the impression procedure for the ocular prosthesis.

Petroleum jelly was applied to the eyebrow, eyelashes and skin around the socket to prevent impression material from sticking to them. A thin mix of alginate impression material was mixed and loaded in a 2 ml plastic disposable syringe. Impression material was slowly injected into the socket. The impression was carefully removed from the socket and checked for any air bubbles. The impression was separated from syringe and invested in type III gypsum stone to make a two-part mold. Molten wax was poured in this mold to obtain the scleral wax pattern. It was tried in the patient and checked for proper contour and retention while performing the various eye movements. For iris positioning, the patient was asked to maintain a straight gaze at an object kept 6 feet away. Shade was selected as per the patient’s normal eye sclera. Flasking was done in a two-part metal flask followed by dewaxing, packing and curing. The retrieved prosthesis was trimmed, polished and inserted. Prior to insertion of the finished prosthesis, it was disinfected using 70% isopropyl alcohol and 0.2% chlorhexidine solution. After thoroughly cleaning the prosthesis with saline solution to prevent chemical irritation, it was inserted and checked for fit, contour, and movements (Figure 3).

Discussion:

Loss of eye has functional, aesthetic and psychological impact on the patient. Rehabilitation of such defects with ocular prosthesis can improve his/her physiological and psychological well-being. An ocular prosthesis should replicate correct gaze, shape, and colour of the natural eye. It should prevent collapse or loss of the shape of the lids, accumulation of fluid in the cavity and provides proper muscular action of the lids. A well-fabricated prosthesis not only restores function and aesthetics but also restore patient’s self-confidence and psychological health. 

2
After enucleation or evisceration, it is necessary to replace the lost volume in the orbit, which is a condition that was recognized since the beginning of the 20th century.4,5 The first orbital implants were produced by Mules in 1885, who used hollow glass spheres to restore the anophthalmic cavity volume. Subsequently, diverse materials were used to manufacture orbital implants; however, the advances in this field progressed relatively slowly until the 1940’s when various materials were suggested.6,7 The most successful were acrylic and silicone spheres, and the use of both has spread throughout the world. Acrylic and silicone implants are both smooth, non-porous, and non-integrated implants, which are inert and they cause little reaction in the host. These are still the most popular implants.8

A revolution in anophthalmic cavity reconstruction occurred when integrated implants emerged in the 1980s to improve the results obtained during anophthalmic cavity treatment, particularly in terms of the mobility of the external prosthesis.9

Unfortunately, many complications are associated with integrated implants. Rates of exposure and postoperative inflammation of integrated Hydroxyapatite implants range from 0% to 22%, while it is almost (0-7%) for acrylic and silicone implants.

Thus, the main reason for using integrated implants with a coupling system between the implants and external prosthesis was to improve mobility. However, numerous complications have been described, which are caused by dehiscence and the exposure of the implants, and they necessitate the removal or extrusion of the integrated implants. Therefore, the pegging system is rarely used at present. However, if pegging is not planned, there is no advantage in terms of mobility when using porous orbital implants instead of solid silicone and acrylic spheres.10

Previous studies provide no evidence that integrated implants are superior to non-integrated implants. The superiority of porous polyethylene has been reported but others note that porous polyethylene has the same rate of complications as other porous or non-porous implants.11

Several case reports indicate that complications have occurred with all of the different types of implants. Randomized studies and long-term follow-up are required to conclusively determine the performance of implants. Therefore, according to the current state-of-the-art and for the implants that exist in the market, it is possible to affirm that there is no ideal implant and that the integrated implants do not perform better than the non-integrated implant.12

An ideal orbital implant and surgical technique should yield excellent prosthesis motility and cosmesis, with very few complications. The technique includes choosing an appropriately sized implant, positioning the implant deeply in the orbit, meticulously closing the Tenon’s capsule over the implant, and securing the conjunctiva over the implant without tension.2

Size, shape, composition and cost determine the ideal type of implant. It must approximate the normal eye, occupy the excess orbital volume and must also be made of a material the body will not reject. Implant shape also plays a role in the mechanics of the prosthesis. It may be spherical or irregularly shaped (pyramidal, conical or egg shaped). We used the traditional spherical shaped implants to simulate the shape of the eyeball.1

Infection is less frequent with nonintegrated than the integrated implants because of the lack of pores which act as areas for potential complications.

The solid acrylic spheres are used commonly due to its low cost.1 The acrylic implant used here has improved the motility of the prosthesis to a great extent. We used the acrylic implant because of its inert properties, low cost and long history of use as an orbital implant.

Considering the Indian economic system and the socio economic status of Indian patients, the acrylic implant is definitely a poor man’s boon compared to the expensive porous implants with a very similar prosthetic motility.

Acknowledgement: I express my profound gratitude to Dr Ajay Kudva, Ophthalmologist at AJ Institute of Medical Sciences, Mangalore for the orbital implant placement.

Ethical Clearance obtained from Institutional Ethical committee, Manipal College of Dental Sciences, Mangalore.

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Effect of Exercise Training Program in Pain, Muscle Strength and Functional Mobility in Post Cervical Decompression Surgeries

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Abstract

Background: Cervical disease is caused by various factors like degeneration, disc protrusion. According to previous studies, there are 20 – 30% patients with cervical disc disease. There are various complications according to the level of lesions. Cervical disc diseases are the condition which affects the motor and sensory components of the body. This study specially emphasis on the sensory and motor components. This study focuses on the stage wise progression of the patient. The patients also have reduced functional ability. This study emphasis on the increasing the functional ability of the patients. Greater efficacy of rehabilitation can be brought about by early intervention for improving strength and increase sensory integration which might achieve activities of daily living goals.

Aim: To find the effect of exercise training program in patients with cervical decompression surgeries.

Methodology: There were 35 participants in the study. The type of study is experimental. The participants involved were assessed for strength, pain and functional mobility. The participants are treated for pain, strength and functional mobility.

Result: There is improvement seen in participants. The results of the participants are calculated by mean and standard deviation. The pre assessment is 56% and post assessment is 38% percentage for pain. The pre assessment is 1.74% and post assessment is 3.31% percentage for functional mobility. The pre assessment is 23.4% and post assessment is 50.8% percentage for right upper limb strength. The pre assessment is 23.2% and post assessment is 42.8% percentage for right lower limb strength. The pre assessment is 23.4% and post assessment is 48% percentage for left upper limb strength. The pre assessment is 23.4% and post assessment is 41.7% percentage for left lower limb strength. The pre assessment is 13.7% and post assessment is 42.8% percentage for trunk strength.

Conclusion: Structured exercises program effective in reducing pain and improving muscle strength and functional mobility in patients with post cervical decompression surgeries.

Keywords: Cervical decompression surgeries, physiotherapy, pain, strength, functional mobility

Introduction

Spinal cord diseases are the conditions were it actually affects the entire body in one or the other way. There is a classification of spinal cord diseases. Diseases are classified on the basis of compressive, vascular, inflammatory, infective, inflammatory, developmental, metabolic, congenital, degenerative, traumatic. Spinal
cord diseases can either cause quadriplegia or paraplegia. Complete lesion is in which all sensations and motor fibers below the level of lesions is lacking\textsuperscript{1}.

According to a survey it has been reported that cervical spine is injured in 2.4\% of blunt trauma victims\textsuperscript{2}. Classification systems have been developed in an attempt to predict instability, standardize the discussion of injury types and provide a means for applying a consistent approach to these injuries\textsuperscript{2}. These are divided into upper cervical spine and sub axial cervical spine\textsuperscript{1,2}. Cervical injury includes cervical disc degeneration, fracture at the cervical region, any neurological problems with nerve compression\textsuperscript{2}.

Cervical decompression surgery is a procedure that removes any structure compressing the nerves in neck\textsuperscript{3}. There are various sub types in cervical decompression surgeries\textsuperscript{3,4}. ACDF includes removal of cervical disc from an anterior approach and then decompresses the nerve\textsuperscript{24,21}. Posterior cervical decompression surgery enlarges spinal canal area, allowing the spinal canal to drift away from ventral compression, however, while doing this it also destabilizes the dorsal structures and can lead to progressive kyphotic deformity\textsuperscript{4,12}. Anterior decompression includes anterior cervical corpectomy\textsuperscript{16,17}. Posterior decompression includes laminoplasty and laminectomy\textsuperscript{3,4}.

Secondary complications are pain, weakness, loss of mobility, reduced range of motion, loss of doing functional activities etc\textsuperscript{4}. Strength of the patient is the most important and the basic requirement for the recovery of the patient\textsuperscript{26}. Strength in turn takes further part in improving range of motion and also to improve the functional mobility\textsuperscript{5}. Functional capacity is to act on, influence or change the surrounding environment has been studied as one important aspect of late adulthood and elderly life\textsuperscript{14}. The functional mobility is related to the physical activity\textsuperscript{5}. Joint range of motion and soft tissue flexibility are important elements of motor function\textsuperscript{5}. Limitation restricts the normal coordinated action of muscles and alter biomechanical alignment of body segments and posture\textsuperscript{6}. The resultant compensatory movement patterns are frequently dysfunctional, producing additional stresses and strain on musculoskeletal system\textsuperscript{6,7}. They do cost more energy consumption and can limit functional mobility\textsuperscript{8}. These are the problems which are to be treated for the betterment of the people. So that the individual should regain their mobility, increases range of motion, increases their strength, activities of daily living and return to the normal living\textsuperscript{10}.

Improvements in performance results from an understanding the task and practice and a frequently used measure of learning\textsuperscript{7}. Resistance exercise has been shown to be safe and effective method for improving strength and additional it improves cardiovascular endurance. A detailed, accurate, and specific determination of functional skills is usually delayed until the active rehabilitation stage when the patient is medically stable and cleared for activity. There are certain exercises given for the patients with pain, muscle weakness and less functional mobility\textsuperscript{8,22}.

In the project there are exercises which focuses on improving pain, muscle and functional mobility. For pain, the patient is treated with transcutaneous electrical stimulation and also ice\textsuperscript{8,25}. Active exercises for neck to reduce pain\textsuperscript{13,20}. Before starting the exercise the patient is started with stimulation. After this the patient is started with passive movement\textsuperscript{9}. This improves the range of motion. As the patient starts the regaining the range, the patient is started with strength training with the weight cuffs, elastic band\textsuperscript{18}. As the patient starts regaining the range and improving strength, the patient also starts get trained for functional mobility\textsuperscript{23}.

There are various studies on cervical decompression surgeries, where there has been treated with pain, muscle strength, range of motion, functional mobility\textsuperscript{15}. But these are treated with the separate aspect\textsuperscript{11}. And many times, the condition does not emphasis on functional training as soon as the muscle strength is gained. But to emphasis on the functional mobility is as important as training muscle strength. Specific trunk exercises including strengthening and conventional have been found to improve strength in paraplegia individuals\textsuperscript{27}. There is no combined structured exercises for treatment. There is a need to emphasis on the complication as a whole and a need to have structured exercise program for the improvement so that the patient gets back to the work as soon as possible\textsuperscript{19}. So the study focuses on the structured exercise program for cervical decompression surgeries. This study is to evaluate the effect of these structured exercise has improvement on pain, muscle strength and functional mobility.

**Material and Method**

There were 35 participants in the study. The study was taken place in Krishna institute of medical sciences.
The study is effect of exercises training program on pain, muscle strength and functional mobility in post cervical decompression surgeries. The treatment protocol was of 6 weeks. The participants were assessed for pain, strength and functional mobility at first. The type of the study is experimental study. Sample size was calculated by the formula $4pq/l^2$. Inclusion criteria is post-operative cervical decompression surgery patients, patients with sensory and motor impairments, age group above 40-60 years. Exclusion criteria is history of previous fracture, spinal infections, post-operative infection and previous spondylodiscitis. Outcome measure used for pain is visual analogue scale, for strength is manual muscle testing and for functional mobility is functional independence measure scale.

**Findings**

The study was taken place in Krishna institute of medical sciences. The results were calculated by the pre and post assessment. The mean and SD were calculated. The significance of the study was calculated. The instat software was used to calculate all the results. The values are calculated by paired ‘t’ test. The P value for pain, muscle strength and functional mobility is <0.0001 which is extremely significant.

**Chart no. 1:** Pre and post changes in pain after cervical decompression surgeries.
Interpretation: the mean and SD of pre pain is less than post pain.

**Chart no. 2:** Pre and post changes in functional mobility after cervical decompression surgeries.
Interpretation: the mean and SD of pre functional mobility is more than post functional mobility.

**Chart no. 3:** Pre and post changes in strength of right upper and lower limb after cervical decompression surgeries.
Interpretation: the mean and SD of pre strength is more than post strength of right upper and lower limb.

**Chart no. 4:** Pre and post changes in left upper and lower limb after cervical decompression surgeries.
Interpretation: the mean and SD of pre strength is more than post strength of left upper and lower limb.
Discussion

The study topic is on effect of exercise training program on pain, muscle strength and functional mobility in post cervical decompression surgeries. The aim of the study is to determine effect of exercise training program on pain, muscle strength and functional mobility in post cervical decompression surgeries. The treatment was given by a fixed protocol. The study was taken place in Krishna institute of medical sciences. The number of participants included were 35. Female included in the study were 15 in number and male included were 20. The participants were taken according to the inclusion and exclusion criteria. The participants were assessed for pain, strength and functional mobility before the treatment. The appropriate treatment for improving pain, strength and functional mobility is given. Treatment protocol was for 6 weeks. A consent was taken by the participants before the treatment. After the treatment again the assessment is taken.

There are various studies on the cervical decompression surgeries. According to a study by author AnneliPeolsson, which was on outcome of physiotherapy on cervical disc disease stated that there are better results of physiotherapy. This study supports the study as the outcome of the study is beneficial for the participants.1

To reduce pain icing is applied. To initiate movements of limbs passive movements are given, followed by active assisted movements then active movements along with electrical stimulation is given. To strengthen the limb, weights and elastic bands are used. And to improve functional mobility bed mobility and gait training is given.

In patients with cervical decompression patients, the intervention strategies selected is largely based on amount of preserved motor function. The pain of the participants is reduced by using ice. Ice is used to reduce pain, as it involves stimulation of cold receptors which passes into the spinal cord via posterior root and effectively blocks pain. Electrical stimulation stimulates the motor nerve. As the muscle contracts due to stimulation, the changes take place similar to those associated with voluntary contraction. The strength of the participants is improved by strength training program. It increases the production of maximal force due to changes in neural drive, increases connective tissue tensile strength, bone mineral density and improve functional performance and activity level.

Functional mobility of the participants is improved. For functional mobility bed mobility was included. This is because functional mobility training represents a shift away from some conventional rehabilitation approaches that utilize an extensive hand on approach to promote recovery. As the strength of the lower limb is regained, gait of the participants is improved. Gait is improved by using parallel bars progressing to the without support of walking. Then followed by stair climbing.

Conclusion

Structured exercises program is effective in reducing pain and improving muscle strength and functional mobility in patients with post cervical decompressionsurgery.

Conflict of Interest: There are no conflicts of interest.

Funding: Funding is given by Krishna institute of medical sciences, “Deemed to be” University.

Ethical Clearance: Ethical clearance was taken from institutional committee of Krishna institute of medical sciences, deemed to be university, Karad.

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A Study on Bio-Medical waste Segregation Monitoring in a Tertiary Care Hospital at Telangana

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Abstract

The most appropriate way of identifying the categories of healthcare waste is by sorting the waste into color-coded plastic bags or containers.¹,² Since early recovery of patients and health of clinical staff directly depends on a clean and hygienic environment, excellent hygiene practices in health care facilities are the prerequisite for good medical waste management.³

Objective: Aim of this study is to see effectiveness of segregation practiced in the hospital and establish a monitoring system to monitor deficiencies in segregation.

Method: This cross sectional study conducted during the period of June to August 2012 in a tertiary care Hospital. Eighty Two (82) areas were identified as BMW generation area. As per the norms segregation was done in four different color coded containers. So, total segregation containers will be 82X4=328. Any mix up or wrong segregation will be noted in the register daily by the Infection Control Nurse. A Monthly report based on BMW Register was developed.

Results: The index named as Bio-Medical Waste Segregation Deficiency index (BMWSD index), calculated as number of deficiencies found in a area/areas divided by the possible number of deficiencies can occur in that area/areas. Daily data then entered into the Monthly report and monthly BMWSD index was made. Though this is a continuous process, for this study three months data (June, July and August 2012) were taken for analysis.

Discussion: In this Cross sectional study, it was found that there was a over all deterioration in segregation of BMW when compare to June, July and August reports. The areas with high BMWSD index or where segregation is poor will be reflected through this monitoring system.

Conclusion: This BMWSD index can be used as an indicator of Infection control Practice. This report is to identify the deficiency areas in the entire hospital in BMW segregation, which can be used for analysis and planning for better BMW Management like, training of the staffs of particular units, modification in methods etc.

Key Words: Waste Segregation, BMW Color coded system, Biomedical Waste Management,

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Introduction

Bio-Medical Waste (BMW) Segregation is one of the most important steps to successfully manage Hospital Waste. Given the fact that only about 10-20% of the hospital waste is hazardous. Treatment and disposal costs could be greatly reduced if a proper segregation were performed. Segmenting BMW from Hospital
waste reduces also greatly the risks of infecting workers handling hospital waste and can be reduced to 2-5% if the hazardous part were immediately separated from the other waste. But, if 1% of the biomedical waste is mixed with the general waste, the whole becomes biomedical waste! Liquid wastes in particular. A worst case scenario of BMW management is in developing countries. 1,2,3 Hospital waste management is a part of hospital hygiene and maintenance activities. World Health Organization4 also states that 85% of hospital wastes are actually non-hazardous, whereas 10% are infectious and 5% are non-infectious but they are included in hazardous wastes. This range is dependent on the total amount of waste generated 5. Since the infectious waste gets mixed with municipal solid waste, it has potential to make the whole lot infectious in adverse environmental conditions6 The hazardous waste is a potential reservoir of the infection and diseases in patients are transmitted through it 7. The key to minimization and effective management of biomedical waste is segregation (separation) and identification of the waste. Since early recovery of patients and health of clinical staff directly depends on a clean and hygienic environment, excellent hygiene practices in health care facilities are the prerequisite for good medical waste management 8.

**Review of Literature**

Bio-medical waste classified as per WHO in nine different categories which includes Radioactive waste. 9,10 But as per Bio-medical Waste (Management and Handling) Rules, 1998; waste are classified into ten different categories without mentioning Radioactive waste.9 Based on Schedule I (Rule 4 and 7) Draft BMW Rules, 2011; Bio- Medical Waste were categorized into eight categories excluding Liquid waste and Incineration ash from 1998 rules. The Kuwaiti Environmental Public Authorities (KEPA) 20 divided the health waste into two categories: Non-hazardous and Hazardous. In few other literatures 11, 12, 13, 14, waste was classified into two namely, contaminated and non-contaminated waste. The unified medical system of the Gulf Cooperation Council Countries, 15 classified hospital waste into eight categories including Radioactive waste and Pressurized gas containers. Another source 16 categorizes the healthcare waste into seven types including radioactive waste and general waste. Total BMW volume generated in the hospitals is 611.5Kg/per day and 18345Kg / per month as found in all the surveyed hospitals in Lagos Metropolis in Nigeria. 17 According to Manyele (2006) 18, BMW generation is high and is increasing in Tanzania reaching up 0.75Kg/bed per day on average. Hospitals generate up to around 8 kg of waste per bed per day in USA, if not properly managed 19. Table shows the average quantity generated by various countries 20, 21, 22, 23,24.

<table>
<thead>
<tr>
<th>Country</th>
<th>Quantity (kg/bed/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>U. K.</td>
<td>2.5</td>
</tr>
<tr>
<td>U.S.A.</td>
<td>4.5</td>
</tr>
<tr>
<td>France</td>
<td>2.5</td>
</tr>
<tr>
<td>Spain</td>
<td>3.0</td>
</tr>
<tr>
<td>India [25]</td>
<td>1.5</td>
</tr>
<tr>
<td>Kuwait</td>
<td>3.8</td>
</tr>
</tbody>
</table>

In a study 26 at Bangalore, India, found that solid waste generated from hospitals and nursing homes generally varies from ½ kg to 4 kg per bed per day in Govt. hospitals, ½ to2 kg per bed per day in Private hospitals, and ½ to 1 kg per bed per day in Nursing homes. Total quantity of hospital waste generated at Bangalore about 40 tons per day; out of it 45-50% infectious waste and segregation of BMW done in only 30% of hospitals 9, 26, 27. Based on hazard Hospital Waste in India can be divided as given in Table.

**Table 2: The basic types of the Hospital wastes in India.**

<table>
<thead>
<tr>
<th>Type of waste</th>
<th>Percentage [21]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non Hazardous</td>
<td>85%</td>
</tr>
<tr>
<td>Hazardous</td>
<td>15%</td>
</tr>
<tr>
<td>Hazardous but not infective</td>
<td>05%</td>
</tr>
<tr>
<td>Hazardous and infective</td>
<td>10%</td>
</tr>
</tbody>
</table>

Another study also says that 80% of the hospital waste are of generated waste and rest of the hospital wastes requiring specific management.28 The approximate chemical composition of health-care waste shows 50% carbon, 20% Oxygen, 6% Hydrogen and rest other elements. 29

The Segregation processes should: 30 Always take place at the source,
Be simple to implement and applied uniformly throughout the hospital;

Be safe and guaranty the absence of mixing in the domestic waste flow;

Be well understood and well known by the staff of the hospitals;

Be regularly monitored to ensure that the procedures are respected.

Mutilation / shredding must be such so as to prevent unauthorized reuse.31

**Indian BMW Legislation Status**

First law related to Bio-Medical Waste, is “The Bio-Medical Waste (management and Handling) Rules, 1998”.9 By this law India is become the one of the country having legal regulation regarding BMW. The said law is in the vicinity of Ministry of Forest and Environment, and executed through Pollution Control Board (PCB). The law has been amended twice in 2000 and 2003.9 A draft Rule has been formulated in 2011.

**Objective**

Aim of this study is to see how effective segregation practiced in the hospital under study. Also, to establish a monitoring system, that can pick up deficiencies in segregation properly and easily and segregation practice can be improved.

**Method**

This cross sectional study conducted during the period of June to August 2012 in a tertiary care super specialty hospital in the major towns of the state of Telangana. Through out the Hospital Eighty Two (82) areas were identified as BMW generation area. As per the norms segregation was done in four different color coded containers. So, total segregation containers will be 82X4=328. Any mix up or wrong segragation will be noted in the register daily by the Infection Control Nurse. A Monthly report based on BMW Register was developed. This report is to identify the deficiency areas in the entire hospital in BMW segregation, which can be used for analysis and planning for better BMW Management like, training of the staffs of particular units, modification in methods etc.

**Results**

Daily Infection Control Nurse, document any deviation in the segregation of BMW as per Rules, in those 328 containers. For every day and for each area a index has made for comparison with other areas and for subsequent future comparison. The index is named as Bio-Medical Waste Segregation Deficiency index (BMWSD index). The index is calculated as number of deficiencies found in a area/areas divided by the possible number of deficiencies can occur in that area/areas. Daily data then entered into the Monthly report and monthly BMWSD index was made. Though this is a continuous process, for this study three months data (June, July and August 2012) were taken for analysis.

First Top ten (10) areas with descending order of BMWSD index is shown in the below table.

**Table 3: Top Ten Areas as per BMWSD index in June, July and August 2012.**

<table>
<thead>
<tr>
<th>S No.</th>
<th>BMWSD Index</th>
<th>BMWSD Index</th>
<th>BMWSD Index</th>
<th>BMWSD Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Casualty/ER</td>
<td>10</td>
<td>Casualty/ER</td>
<td>9.677</td>
</tr>
<tr>
<td>2</td>
<td>7th GW - B</td>
<td>10</td>
<td>2nd C</td>
<td>6.451</td>
</tr>
<tr>
<td>4</td>
<td>Urodynamics</td>
<td>6.666</td>
<td>Block – C</td>
<td>6.451</td>
</tr>
<tr>
<td>5</td>
<td>Block - C</td>
<td>6.666</td>
<td>7th RAS</td>
<td>6.451</td>
</tr>
</tbody>
</table>
Below is the representation of the entire hospital Avg. BMWSD index calculated taking average of 82 areas BMWSD indices for the three month

<table>
<thead>
<tr>
<th>Rank</th>
<th>Area</th>
<th>BMWSD Index</th>
<th>Area</th>
<th>BMWSD Index</th>
<th>Area</th>
<th>BMWSD Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>7th RAS</td>
<td>6.666</td>
<td>9th –A</td>
<td>6.451</td>
<td>Sample Collection -I</td>
<td>3.225</td>
</tr>
<tr>
<td>7</td>
<td>9th –B</td>
<td>6.666</td>
<td>9th –C</td>
<td>6.451</td>
<td>ENT OP</td>
<td>3.225</td>
</tr>
<tr>
<td>8</td>
<td>9th –C</td>
<td>6.666</td>
<td>Dressing Room</td>
<td>3.225</td>
<td>2nd C</td>
<td>3.225</td>
</tr>
<tr>
<td>9</td>
<td>Dental OP</td>
<td>3.333</td>
<td>Sample Collection –I</td>
<td>3.225</td>
<td>ICCU - II</td>
<td>3.225</td>
</tr>
<tr>
<td>10</td>
<td>2nd C</td>
<td>3.333</td>
<td>Sample Collection –II</td>
<td>3.225</td>
<td>OT - 4</td>
<td>3.225</td>
</tr>
</tbody>
</table>

Along with these, at a glance different area performing poorly will be identified and the probable reasons for the loopholes were searched through the interview with the corresponding unit in-charges and local observations listed at end. This will help to take necessary Corrective and Preventive Actions (CAPA).

**DISCUSSIONS:**

In this Cross sectional study, it was found that there was a deterioration in segregation of BMW when compare to June, July and August reports. This shows a gradual deterioration in BMW segregation. The BMW segregation (BMWSD) index of overall Hospital was increase in July (1.219) as compared to June (1.138) and again goes down in August (1.180) but still higher than June 2012. Which means the BMW segregation was good at in June but deteriorates in July and again improves a little in August as shown in Figure 1. In terms of generation areas, Casualty/ER shows a continuous improvement in segregation of BMW Management. BMWSD index in June (10) to in July (9.67) to in August (9.45). Similarly, in the generation area of 2nd C there is a deterioration in BMW segregation in July (6.45) as compare to June (3.33) and again improve in August (3.2). Also it can be seen that in the generation areas of 9th C, there was improvement in segregation of BMW (from 6.6 to 6.45) and in Sample Collection -I there was constant deterioration in BMW segregation (from July to August at 3.22).

Though only three months data has been taken for analysis, it is a continuous process. The areas with high BMWSD index or where segregation is poor will be reflected through this monitoring system. As shown in above in casualty/ER for the month of June (BMWSD -10) was high which after training the staff is gone down in August (BMWSD - 9.45).

**Conclusions**

Segregation of BMW is the prime and first step in the Bio Medical Waste Management. This study revealed that the BMW management requires proper monitoring for an effective tracking at all times. This BMWSD index can be used as an indicator of Infection control Practice. Control means that competent authorities can act rapidly to ensure the possibilities of minimizing inappropriate handling and segregation of BMW. Also there is an immediate need to train all handlers of BMW.
on methods and new techniques to adopt in effective waste management practices using WHO manual and Personal Protective Equipment (PPE). Furthermore, they should also be trained on how to make proper use of these PPE.

**Recommendations**

We recommend the following suggestions to improve BMW management service in this hospital under study from the point of view of Monitoring study of BMW segregation:

More Strict supervision and surveillance to be followed in day to day BMW management activities.

Intensive training and displayed information regarding risk associated to BMW on BMW at regular interval and also at the time of joining to all staff emphasizing the importance of segregation.

**Ethical Clearance:** Ethical clearance had been obtained from Multispeciality hospital, Telangana

**Conflict of Interest:** NIL

**Source of Funding:** Self

**References**


Effectiveness of Open Kinematic Chain Exercises and Closed Kinematic Chain Exercises in Adductor Groin Pain in Recreational Football Players

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Abstract

Background: Football has been growing considerably for the past decade in India. Many young adults have taken up football as their passion. However, the grounds are underdeveloped with limited number of facilities required for a safe game. Coaching facilities are inadequate and thus fail to provide basic information about the precautions to be taken during play or the necessary steps to be taken post injury. This leads to increased risk of injuries. Groin injuries comprise of 10-18% of all football injuries.

Objective: The objective of the study was to compare the effects of open and CKC exercises on groin injuries in young adults who play football for recreation. Pain Assessment, Tenderness assessment and MMT were used for assessing the individuals.

Method: There were total 40 subjects who were willing participants of this study of which 3 dropped out. This was a study conducted to find the effects of open and CKC exercises on groin injuries in young adults who play football for recreation. Here we evaluated the pain using VAS and tenderness grading. Muscle strength was assessed using MMT.

Result: There was a significant effect of open and CKC exercises on groin injuries. There was a reduction of pain; both on activity as well as on resistance (p=0.0065 and 0.0235) respectively. Tenderness was also less (p= 0.0130). Muscle strength did not show any significant change (p= 0.1775)

Conclusion: There is a significant effect of OKC and CKC exercises being administered together rather than giving only CKC exercises on reducing pain and reducing the risk of injuries in recreational football players.

Key Words: Recreational, Football, Groin Injury, Pain, Tenderness, MMT.

Introduction

Football is a sport that has been widely accepted in our country. Many young adults have taken up this sport. However most of the players only play for recreation. Such recreational players are those who play only for enjoyment and not to compete in any leagues.¹

The term ‘adductor related groin pain’ first originated in the literature in 1997.

Groin injuries are defined as any physical symptom in the groin related to participation in soccer training or match play, incapacitating the player while playing soccer or demanding special medical attention.²

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Groin injuries occur most commonly in non-contact situations (71%). Here, a rapid muscle contraction during rapid muscle lengthening is the fundamental mechanism. A quick reaction to a change in play, such as sudden change in direction, running, kicking etc. were the common modes of injury.6

Groin injuries make up 2-5% of all sports-induced injuries and 10-18% of all football injuries.

Prevalence of groin injuries in male cub football is 4-19% of all injuries. It was reported in 2002, that about 9.5% of all male football players had groin injuries.4,7

Despite groin injury being one of the most prevalent injuries in soccer, it poses a great amount of trouble to a sports physiotherapist. They are extremely difficult to treat, often presenting with long term symptoms8,9, unclear prognosis8, long period of absence10 from play and a high rate of recurrence11. This is further added to, by a lack of knowledge among the population about how to approach this subject as it can be a sensitive topic for some. Thus many players just ignore the symptoms which leads to worsening of the symptoms overtime making it more difficult to treat groin injuries.

The treatment of choice for groin injury is physiotherapy.10 Many forms of therapeutic exercise are chosen as treatment for groin injury, of which OKC and CKC exercises are frequently used.

OKC exercises are non-weight bearing exercises as they involve movement around a single joint. The distal segment is the focus of these exercises. This segment is free to move and also resistance is applied here. An example for the same is when resistance is applied over the ankle in seated knee extension. There is sequential activation of muscles, from proximal to distal, allowing rapid acceleration and speed of movement.12,13

CKC exercises are generally weight bearing. Here movement occurs at several joints and no joint is really free to move. Both the distal and the proximal joints are fixed and resistance is applied. Resistance can be applied to both the proximal and/or the distal segment. An example of this is a squat. These exercises imbibe early stability in the proximal joint.12,13

A review of various studies shows that the optimal treatment for long standing adductor groin injury is therapeutic exercise.8,9,10,11,14 A study by Hölmich et al. (1999), concluded that hip and abdominal strengthening exercises along with physiotherapy such as laser therapy, TENS, stretching and transverse friction massage lead to better pain reduction and quicker return to sports.8,9,15 However in Hölmich’s study, the average time taken from the beginning of the treatment to the subject’s return to sport was approximately 18.5 weeks. Unfortunately, such a long duration of recovery is not feasible for an athlete, especially elite as there is a lot of pressure on them to return back to their sports in the shortest period of time possible.13,16,18

Hence, it is necessary to create an exercise protocol that will benefit these recreational players and will in turn improve their performance and reduce their risk of injury.

Methodology

Included participants were divided in 2 groups (group A and group B) by convenience sampling method. Group A consisted of 19 players and group B consisted of 18 players. Demographic data was acquired. They were explained the purpose of the study. Pre and post assessment was taken with the help of the outcome measures.

Procedure

Group A received ckc and okc exercises 4 times a week for 5 weeks.

For the 1st and 2nd week leg press, squats with medicine ball between the legs, lunges, side-lunges, Resisted seated hip adduction, Resisted standing hip abduction, Resisted standing hip adduction, Supine leg abduction adduction and Resisted side lying hip adduction for 10 repetitions for a 5 sets. For the 3rd and 4th week they were for 20 repetitions for 5 sets. For the 5th week they were for 30 repetitions for 5 sets.

Group B received ckc exercises 4 times a week for 5 weeks.

For the 1st and 2nd week leg press, squats with medicine ball between the legs, lunges, side-lunges, for 10 repetitions for 5 sets. For the 3rd and 4th week they were for 20 repetitions for 5 sets. For the 5th week they were for 30 repetitions for 5 sets.

Result

1) Age wise distribution:
Table 1: Age wise distribution

Interpretation: Table no. 1 shows that, the mean age of individuals in Group A and B

2) VAS score

Table 2: VAS score

<table>
<thead>
<tr>
<th>Mean VAS Score (VAS)</th>
<th>Group A</th>
<th></th>
<th>Group B</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>On Activity</td>
<td>On Resistance</td>
<td>On Activity</td>
<td>On resistance</td>
</tr>
<tr>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>5.43±0.91</td>
<td>0.83±0.82</td>
<td>7.07±0.69</td>
<td>0.92±0.93</td>
<td>5.82±0.90</td>
</tr>
<tr>
<td>6.82±0.62</td>
<td>1.23±0.86</td>
<td>1.23±0.86</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Interpretation: Table no. 2 shows that, the mean VAS scores on activity and on resistance for group A and B

3) Tenderness and MMT:

Table 3: Tenderness and MMT

<table>
<thead>
<tr>
<th>TENDERNES</th>
<th>Group A</th>
<th>Post</th>
<th>Group B</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.28±0.46</td>
<td>0.5±0.51</td>
<td>1.37±0.49</td>
<td>0.95±0.52</td>
<td></td>
</tr>
<tr>
<td>MMT</td>
<td>4.72±0.46</td>
<td>4.94±0.23</td>
<td>4.68±0.47</td>
<td>4.79±0.41</td>
</tr>
</tbody>
</table>

Interpretation: Table no. 3 shows that, the mean tenderness and MMT grades for Group A and B pre and post treatment

4) Wilcoxon signed ranks test and paired t-test results

Table 4: Wilcoxon signed ranks test and paired t-test results

<table>
<thead>
<tr>
<th>VAS w- value</th>
<th>Wilcoxon signed ranks test</th>
<th>p- value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A</td>
<td>On Activity 171.0&lt;0.0001(ES)</td>
<td>&lt;0.0001(ES)</td>
</tr>
<tr>
<td></td>
<td>On Resistance 171.0&lt;0.0001(ES)</td>
<td>&lt;0.0001(ES)</td>
</tr>
<tr>
<td>Group B</td>
<td>On Activity 190.0&lt;0.0001(ES)</td>
<td>&lt;0.0001(ES)</td>
</tr>
<tr>
<td></td>
<td>On Resistance 45.0 0.0039(VS)</td>
<td></td>
</tr>
</tbody>
</table>

Paired t-test

<table>
<thead>
<tr>
<th>TENDERNES</th>
<th>t- value</th>
<th>p- value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>4.507</td>
<td>0.0003(ES)</td>
</tr>
<tr>
<td>Group B</td>
<td>3.618</td>
<td>0.0020(VS)</td>
</tr>
<tr>
<td>MMT</td>
<td>t- value</td>
<td>p- value</td>
</tr>
<tr>
<td>Group A</td>
<td>2.557</td>
<td>0.0204(S)</td>
</tr>
<tr>
<td>Group B</td>
<td>1.821</td>
<td>0.0774(NQS)</td>
</tr>
</tbody>
</table>
**Interpretation:** Table no. 4 shows that, the association between the outcome measures pre and post treatment

5) **Mann-Whitney test and Unpaired t-test**

**Table 5: Mann-Whitney test and Unpaired t-test**

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Group A vs Group B</th>
<th>Mann-Whitney Test</th>
<th>Unpaired t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>U'-value</td>
<td>p-value</td>
</tr>
<tr>
<td>VAS</td>
<td>On activity (Pre)</td>
<td>225.0</td>
<td>0.1037 (NS)</td>
</tr>
<tr>
<td></td>
<td>On activity (post)</td>
<td>261</td>
<td>0.0065 (VS)</td>
</tr>
<tr>
<td></td>
<td>On resistance (pre)</td>
<td>201</td>
<td>0.3696 (NS)</td>
</tr>
<tr>
<td></td>
<td>On resistance (post)</td>
<td>246</td>
<td>0.0235 (S)</td>
</tr>
<tr>
<td>TENDERNESS</td>
<td>Tenderness (pre)</td>
<td>0.5753</td>
<td>0.5688 (NS)</td>
</tr>
<tr>
<td></td>
<td>Tenderness (post)</td>
<td>2.618</td>
<td>0.0130 (S)</td>
</tr>
<tr>
<td>MMT</td>
<td>MMT (pre)</td>
<td>0.2461</td>
<td>0.8070 (NS)</td>
</tr>
<tr>
<td></td>
<td>MMT (post)</td>
<td>1.376</td>
<td>0.1775 (NS)</td>
</tr>
</tbody>
</table>

**Interpretation:** Table no. 5 shows the association between outcome measures pre and post treatment

**Discussion**

Pain assessment was used to note the severity of groin pain in all the participants. The discomfort or pain experienced by the players was divided into 2 categories; pain on activity and pain on resistance. Pain was assessed both before and after completion of the treatment protocol.

Intra-group association was studied using Wilcoxon signed ranks test for VAS and Paired t-test for tenderness and MMT. The results of current study indicate that the changes in groin pain observed from week 1 to week 5 after administering treatment for groin injury differed significantly in groups trained using OKC and CKC exercises versus only CKC exercises. Pain on activity and on resistance within group A post intervention had a p-value of <0.0001 whereas in group B the reduction in pain on resistance was not as significant; seen by the p-value of 0.0039 (VS).

Tenderness was assessed by palpation of the groin pre and post intervention. There was a more significant reduction in tenderness within group A after treatment compared to group B as seen by the p-value of <0.0003 (ES) and 0.0020 (VS) respectively.

MMT was checked by resisting adduction of the hip pre and post intervention. MMT grades again showed a better result within group A compared to group B having a p-value of 0.0204 (S) and 0.1628 (NS).

Thus, the intra-group results following treatment showed to be more significant for group A as compared to group B in terms of reducing pain, tenderness and improving strength.
Also, inter-group comparison between the results of group A and group B was done by Mann-Whitney test for VAS and Unpaired t-test for tenderness and MMT, which proved that the difference in the outcomes of group A and group B was significant, thus confirming that administration of both OKC and CKC exercises together had a better result than administration of CKC exercises only. This is confirmed by the p-value of 0.0065(VS) for pain on activity, 0.0235(S) for pain on resistance, 0.0130(S) for tenderness and 0.0774(NQS) for muscle strength.

Similar studies previously, have showed results supporting our study. A study conducted by Mikkelsen C, Werner S, Eriksson E. investigated the effect of CKC quadriceps rehabilitation versus the same program with addition of OKC exercises following ACL reconstruction on anterior knee laxity and isokinetic muscle torque. They concluded that the addition of OKC quadriceps training after ACL reconstruction results in a significantly better improvement in quadriceps torque without reducing knee joint stability at 6 months and also leads to a significantly higher number of athletes returning to their previous activity earlier and at the same level as before injury.  

When performing OKC exercises, the movement causes a rotatory stress on the joint. Also, they focus on a single muscle group. Thus, a combination of OKC and CKC exercises are better because of use of isokinetic training, which allows specific eccentric loading. Since, most of the movement occurring at the hip in football players is rotatory, leading to groin injury, this may be why addition of OKC exercises to the standard CKC exercises improves groin injury significantly.

Another study by Tagesson S, Öberg B, Good L, Kvist J assessed the impact of a CKC protocol versus that of a OKC protocol on knee pain in subjects post-ACL reconstruction. They concluded that people with ACL deficiency may need addition of OKC exercises to improve quadriceps torque.

OKC exercises have certain biomechanical advantages such as an increase in the rotational forces as well as an increase in the concentric acceleration and eccentric deceleration forces. This leads to a faster increase in muscle strength which is supplemental to CKC exercises in rehabilitation post injury. Hence, this may be a reason for greater improvement in those who received both OKC and CKC exercises.

There are numerous possible reasons for our results. It is important to first keep in mind all the sources of groin pain during the rehabilitation period. Various sources of pain will react differently to our treatment. As we know, the main muscle injured in adductor type of groin injury is the adductor longus. The main movement during OKC activation is hip adduction whereas during CKC activation it acts as a pelvis stabilizer, lower extremity stabilizer during the stance phase and also as a secondary hip rotator. Administration of both OKC and CKC exercises is therefore necessary as the muscles are trained in both the phases of activation separately. This improves the stability and strength of the adductors and the surrounding ligaments leading to a reduction in pain which is comparatively greater than the effect of administration of CKC exercises only.

During the phases of kicking a ball, according to Charnock BL et al, the adductor longus remains at a risk of injury for around 65% of the swing phase. Also, its maximal co traction is seen during the initial 455 of the swing phase and then lengthening occurs. The OKC and CKC exercises administered in our study cover the entire range of a football kick. This improves the strength of the muscles during the kicking motion thus reducing the risk of injury.

Thus, from the above mentioned effects of OKC and CKC exercises being administered together, we can state that they are more effective when administered together than just CKC exercises.

Conclusion

On the basis of the results of the study, it was concluded that there is a significant effect of OKC and CKC exercises being administered together rather than giving only CKC exercises in recreational football players.

Conflict of Interest: There were no conflicts of interest in this study

Ethical Clearance: Ethical clearance was taken from institutional committee of Krishna Institute of Medical Sciences, Deemed to be University, Karad.

Source of Funding: Krishna Institute of Medical Sciences Deemed to be University, Karad

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1) Fuller C.W., et al. “Consensus statement on injury


Self Care Practices among Hypertensive Patients with the View to Develop Informational Pamphlet

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Abstract

Background: Hypertension is condition approximately 25% affecting to adult population worldwide. Hypertension is said to be most common health problem all over the world. Prevalence of hypertension in India was 29.8%.

Objective: study investigated the effectiveness of Informational Pamphlet on Self Care Practices among Hypertensive Patients.

Method: One group pretest posttest design was used to conduct the study among hypertensive patients totally 60 patients were selected by Purposive sampling technique. Study conducted at Krishna hospital Karad. Data was collected by using structured questionnaire. Pre-test was conducted to assess the existing knowledge of self care practices regarding hypertension. Informational pamphlet was given to the patients then posttest level of knowledge was assessed by using same questionnaire.

Results: Results of the present study shows that in the pretest most of the samples were having average knowledge (70%) regarding self care practices regarding hypertension and in postest majority (99.66%) were having good knowledge. The pretets mean was 12.08 and post-test mean was 18.78 and calculated t value was 6.70 found significant at the level of p<0.0001.

Conclusion: Study concluded that information pamphlet was found effective to improve the Knowledge regarding self care practices among hypertensive patients.

Keywords: Self Care Practices, Hypertensive Patients and Informational Pamphlet

Introduction

Hypertension (HTN) or high blood pressure, also called arterial hypertension, is a chronic and common medical condition peoples often suffer. A person with HTN in which the blood pressure in arteries is elevated. High blood pressure is present if blood pressures range at or above 140/90 mm Hg.¹ Cardiovascular diseases are the most common causes of mortality and morbidity in both developed and developing countries.² Hypertension is condition approximately 25% affecting to adult population worldwide.³ Hypertension is said to be most common health problem all over the world. Prevalence of hypertension in India was 29.8%. The patients with stroke 57% deaths and 24% of all coronary heart disease (CHD) deaths in India due to the cause of Hypertension.⁴

In another studies it is estimated that about 31.1% of the world adult population living with hypertension, and 28.5% are in high-income countries while 31.5% are in low-and middle-income countries.⁵ Hypertension is responsible for risk factor for renal and eye diseases.⁶ Increasing awareness regarding hypertension and more effective treatment of patients with hypertension is the main focus of primary prevention of cardiovascular diseases.⁷ Management of hypertension relies on patient’s level of understanding of their condition and treatment and lifestyle changes and pharmacological treatment.⁸

Various studies have confirmed that the self-care practices are important for blood pressure control and reduction of further hypertension complications of cardiovascular and renal diseases. Most of the
hypertensive patients many times do not implement self-care practices and in the end they suffer from uncontrolled blood pressure. Self-care practice regarding hypertension includes medication taking at proper time, consumption of low-sodium and low-fat diet, exercise regularly, less alcohol drinking, not smoking, weight reduction, self-monitoring blood pressure, regular follow-up, and management of stress. In self-care programs, patients become aware about their own health conation, aware about when they need care, and gain adequate knowledge regarding the mode of treatment. Patients are able to monitor their symptoms and they do in time routine examinations without needing to refer to their physician. Health professionals can play a critical role in training general population about self-care activities.

Many of people in India ignore the symptoms like hypertension. Because of the ignorance and their lifestyle, in future they faces problems like acute myocardial infarction, angina pectoris, parallelize stroke, coronary thrombosis etc. and they get hospitalized. Then they realize, the present condition is due to the ignorance of small symptoms which were early observed. Thus it is very important to diagnose the early symptoms to avoid letter complication. Despite of different treatment & lifestyle intervention optimal control of BP remains challenge for many patients. Effective management of HTN depends on patient understanding of their condition & treatment and adherence to lifestyle & pharmacological treatments. Effective patient education material is feasible and widely transferable first step in promoting this goal. Thus the pamphlet should provide information reflecting the latest HTN guideline to provide a better quality life.

**Method**

One group pretest posttest desing was used to conduct the study among hypertensive patients totally 60 patients were selected by Purposive sampling technique. The samples included in this study were who fulfilled the inclusion criteria with who were willing to participate in the study, those who were suffering from hypertension and able to read and understand Marathi language Ethical permission was obtained before the data collection. After obtaining permission from the setting, the patients were asked their willingness to participate in the study and informed consent was obtained. Study conducted at Krishna hospital Karad. Data was collected by using structured questionnaire. Pre-test was conducted to assess the existing knowledge of self care practices regarding hypertension. Informational pamphlet was given to the patients then posttest level of knowledge was assessed by using same questionnaire.

**Statistical analysis used:** To compare the two means Paired t test was used and to find out association between demographic variables and pretest knowledge score Chi Square test was used.

**Results**

**Description of sample characteristics:**

Maximum number 25(41.66%) of Samples belongs to the age group of 46-60 years, 19(31.66%) Samples belong to the age group of 61-75 years. Majority of Samples 39(65%) belongs to male gender group and 21(35%) A sample belongs to female gender group. Maximum number of Samples 25(41.66%) belongs to secondary education, 18(30%) Samples belong to graduate education. Majority number of Samples 49 (81.66%) belongs to Hindu religion, 6 (10%) belongs to Christian religion, 3(5%) belongs to Muslim religion. 

Majority of Samples 40(66.66%) belongs to rural area, 20(33.33%) belongs to urban area. 

Maximum number of Samples 39(65%) belongs to their income of less than Rs.10000. Majority of Samples 22(36.66%) belongs to housewife, 17(28.33%) were doing job Maximum number of samples 60(100%) had got the information regarding their disease from the book.

**Knowledge of self care Practices among Hypertensive patients:**

Graph no.1 shows that majority of the samples (70%) had average knowledge level, (21.66%) had good knowledge level, (8.33%) had poor knowledge level in pretest whereas in the postest majority of the samples (99.66%) had good knowledge level, (3.33%) had average knowledge level, (0%) had poor knowledge regarding self care Practices.

**Effectiveness of Informational Pamphlet on Knowledge Regarding Self-Care of Hypertension:**

Table no. 1 shows that the mean and standard deviation of knowledge score obtained before and after the administering the Informational Pamphlet. This is considered to be extremely significant improvement in knowledge regarding self care practices. The pre-
The test mean was 12.08 and post-test mean was 18.78 and calculated t value was 6.70 found significant at the level of p<0.0001.

The association between pre-test knowledge score and selected demographic variables:

Table no.2 shows the association of pre-test knowledge level of hypertensive patients with their selected demographic variables, using chi square test. There was no significant association found between the knowledge level of hypertensive patients with Age of (2.923), Gender (0.2177), Residence (5.136), & Occupation (10.970). There was significance association between Education (19.627), Religion (16.244), and Monthly income (17.284).

Graph no. 1 Frequency and Percentage Distribution of pretest and posttest Knowledge Score

Table No.1 pre test and post test mean Score, SD and computed t test

<table>
<thead>
<tr>
<th>Phase</th>
<th>Mean</th>
<th>SD</th>
<th>t value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre Test</td>
<td>12.08</td>
<td>3.82</td>
<td>6.70</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Post Test</td>
<td>18.78</td>
<td>1.52</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table no. 2 Association between pre-test level of knowledge and their selected demographic variables.

<table>
<thead>
<tr>
<th>Demographic Variables</th>
<th>Good</th>
<th>Average</th>
<th>Poor</th>
<th>Chi Square</th>
<th>P Value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25-30</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31-45</td>
<td>3</td>
<td>8</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>46-60</td>
<td>5</td>
<td>16</td>
<td>4</td>
<td>2.923</td>
<td>0.9391</td>
<td>NS</td>
</tr>
<tr>
<td>61-75</td>
<td>4</td>
<td>14</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Above 75-</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


**Discussion**

Results of the present study shows that in the pretest most of the samples were having average knowledge (70%) regarding self care practices regarding hypertension and in postest majority (99.66%) were having good knowledge. The pretets mean was 12.08 and post-test mean was 18.78 and calculated t value was 6.70 found significant at the level of p<0.0001.

There was significance association between Education
Religion and Monthly income with knowledge score at the level of p<0.05. The findings of different studies also indicate that self care practices were improved after providing education to the patients. a study conducted by Babaee Beigi MA et al. the mean knowledge scores improved from 2.77 ± 2.7 to 7.99 ± 1.78 after 3 months (P < 0.001). Also, the mean lifestyle scores changed from 3.15 ± 1.52 to 4.53 ± 1.23 (P < 0.001) the study indicated that the educational programs were effective in increasing knowledge, improving self-management, and controlling detrimental lifestyle habits of the patients with hypertension.13 Study conducted by Ademe S et al. the mean score for hypertension self-care was 37.7±8.2 and 51% scored below the mean. Divorced participants (AOR=0.115, 95% CI=0.026, 0.508, p-value<0.01) and those who lack source of information (AOR=0.084, 95% CI=0.022, 0.322, p-value<0.01) were less likely to have good self-care practice. Most of the study participants reported poor self-care practices.14 The education to the patient with hypertension is such a important factor to improve their self care practices related to the hypertension and it will help them to prevent further complications.

Conclusion

Based on the analysis of the findings, the study concluded that information pamphlet was found effective to improve the Knowledge regarding self care practices among hypertensive patients.

Acknowledgement: Our sincere thanks goes to all the study participants who have provided us their valuable time and willingness to participate in the study.

Conflicts of Interest: There are no conflicts of interest.

Financial Support and sponsorship: Nil

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To Determine Tobacco Usage and Associated Risk Factors among Adolescents- A Survey

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Abstract

Aim: To determine tobacco usage and associated factors among adolescents.

Material & Method: The present study was conducted on 1350 students age ranged 12- 17 years of age. Parameters such as type of school, type of tobacco use, type of family, grade, father occupation, mother occupation, monthly pocket money, history of parental use of tobacco and type of family was recorded.

Results: Age group 12-14 years had 780 and 15-17 years had 570 subjects. Subjects were from private school in 890 and government in 460. Grade was 7th in 110, 8th in 230, 9th in 450 and 10th in 560 subjects. Family of subjects was nuclear in 960 and joint in 390. Father occupation was service in 362, farmer in 290, business in 446 and laborer in 252, mother occupation was housewife in 440 and working in 910, parental tobacco usage habit was present in 830 subjects. The difference was significant (P< 0.05).

Conclusion: Authors found that later age, male gender, nuclear family, monthly income >5000 per month, parental use and private school was predictor and risk factor of tobacco usage among school children.

Key words: Tobacco, nuclear family, occupation, Parental use, school children

Introduction

The tobacco use among the young population is on rise. It has been found that an average of 80 thousand to 1 lakh youth start smoking any time. Most of the youth start smoking before 18 years of age.1

Chronic tobacco usage can be credited to sudden immature decision to experiment tobacco during the early adolescent period.2 The harmful effect of tobacco is more when it is stared at early childhood. Both smoking and smokeless forms are frequently used among adolescent. The most common reason for such high may be attributed to marketing strategies of various tobacco companies and its weak regulation.3 Excessive tobacco production, weak enforcement of tobacco control, easy accessibility to child led to increase use of tobacco among adolescents. Modernization, status symbol and social media are other etiological agents.4

Child are more prone to develop addiction and stay proximate till late adult life. Tobacco has the power to kill 250 million of children today a person every 6.5 second globally.5 The harmful effect of tobacco such as smokers melanosis, tobacco pouch keratosis, leukoplakia and oral cancer can be seen among tobacco users. There have been evidences linking youth-smoking with depression,
anxiety, and stress. Global Adult Tobacco Survey for Karnataka stated that the average age for beginning into daily usage of tobacco is 18.7 years.6 The present study was conducted to determine tobacco usage and associated factors among adolescents.

Materials & Method

This study was carried out in the department of Oral medicine. It comprised of 1350 student’s age ranged 12-17 years of age. This study was permitted from institutional ethics committee of Kalinga Institute of Dental Sciences, Orissa. Written consent of all participating subjects was obtained before the study.

Participants’ data such as name, age, gender was recorded in proforma. Other parameters such as type of school, type of tobacco use, type of family, grade, father occupation, mother occupation, monthly pocket money, history of parental use of tobacco and type of family was recorded. Data thus obtained were tabulated and entered in MS excel. Frequency was recorded. Statistical evaluation was carried out using SPSS version 20.0 using Binary logistic regression and Mann-Whitney U-test to detect significant differences for non-parametric data. The P value lesser than 0.05, was considered significant.

Results

Table I shows that multivariate analysis showed that tobacco use was associated male gender (OR: 10.2; 95% CI 8.6-12.4), late age 15-17 years (OR: 1.62 95% CI 1.14-2.16), private school (OR: 1.74 95% CI 0.98-1.84), nuclear family (OR: 0.72 95% CI 0.51-2.1), pocket money/ month (OR: 1.42 95% CI 1.06-2.04) and parental tobacco use (OR: 1.14 95% CI 1.02-1.28).

Graph I shows that type of tobacco usage was cigarette in 45% followed by pan masala in 22%, gutkha in 14%, bidi in 12% and chaini khaini in 7%. The difference was significant (P<0.05).

Graph II shows that reason for tobacco use among students found to be habit in 35%, status symbol in 24%, social media usage in 20%, 15% had misconception that it does not cause harm in and 6% found it to be stress reliever. The difference was significant (P<0.05).

Table I Association of different variables with tobacco use

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>10.2 (8.6-12.4)</td>
</tr>
<tr>
<td>Female</td>
<td>1</td>
</tr>
<tr>
<td>Age group</td>
<td></td>
</tr>
<tr>
<td>12-14</td>
<td>1</td>
</tr>
<tr>
<td>15-17</td>
<td>1.62 (1.14-2.16)</td>
</tr>
<tr>
<td>School</td>
<td></td>
</tr>
<tr>
<td>Private</td>
<td>1.74 (0.98-1.84)</td>
</tr>
<tr>
<td>Government</td>
<td>1</td>
</tr>
<tr>
<td>Type of family</td>
<td></td>
</tr>
<tr>
<td>Joint</td>
<td>1</td>
</tr>
<tr>
<td>Nuclear</td>
<td>0.72 (51.2-101.4)</td>
</tr>
<tr>
<td>Pocket money/ month</td>
<td></td>
</tr>
<tr>
<td>&lt;5000</td>
<td>1</td>
</tr>
<tr>
<td>&gt;5000</td>
<td>1.42 (1.06-2.04)</td>
</tr>
<tr>
<td>Parental tobacco Use</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>1.14 (1.02-1.28)</td>
</tr>
<tr>
<td>Absent</td>
<td>1</td>
</tr>
</tbody>
</table>

Mann-Whitney U-test, Significant, P<0.05

![Graph I Type of tobacco usage](image)
Graph II Reason for tobacco usage

Discussion

Tobacco usage among adolescent in on rise and it is becoming pandemic. The high rate of tobacco related diseases such as moth cancer, stomach cancer, esophagus cancer, etc. is a matter of worry. It has been observed that those who start tobacco use early is life like school age are ore like to continue it for longer life. Tobacco is used in two forms, smokeless and smoking. Under smokeless form, pan masala, chaini khaini, gutkha, betel quid, snuff, mawa are common forms and in smoking tobacco, hooka, hukli, cigarette, bidi, chillum, chutta and dhumti are commonly used forms. The present study was conducted to determine tobacco usage and associated factors among adolescents.

In present study we enrolled 1350 students of which male were 1040 and female were 310. We found that maximum students were from age group 15-17 years (570) followed by 12-14 years (780). Duko et al assessed the prevalence of cigarette smoking among 564 school students by using Global Youth Tobacco Survey (GYTS). They observed that the student prevalence of cigarette smoking was established to be 11% of which 9.4% were current smokers. The prevalence of smoking among male was 8.2% and female students was 2.8%. Students having friends who smoke, khat chewing, age >18 years, alcohol consumption and illegal or illicit drug use were found to be significantly associated with cigarette smoking.

We found that maximum number of students (890) were from private school and grade 10th (560 subjects). Maximum students had habit of tobacco chewing those who were from nuclear family (960). Maximum students’ father occupation was service (362), mother occupation was working (910) and parental tobacco usage habit was present in 830 subjects. Dereje et al evaluated the prevalence of cigarette smoking among adolescents and found that 28.6% students were ever smoker, and 17.2% were current smokers. It was found that gender, alcoholism, parental tobacco usage, peer smoking, exposure to movie with actors smoking, not being exposed to anti-smoking media messages, not discussing in the class about ill effect of smoking.

We observed that tobacco use was associated male gender, late age 15-17 years, private school, nuclear family, pocket money/month and parental tobacco use. In present study cigarette smoking was present in 45% pan masala in 22%, gutkha in 14%, bidi in 12% and chaini khaini in 7%. George et al in their study found that out of the 407 children, 102 had tried cigarette smoking, while 7.9% had tried smokeless tobacco. It was seen in 14.3% of them bought it directly from shopkeepers. Majority of participants thought that smoking can definitely cause harm to health, while others conscious about the harmful effects of passive smoking.

We found that reason for tobacco use was habit in 35%, status symbol in 24%, social media usage in 20%, 15% had misconception that it does not cause harm in and 6% found it to be stress reliever. Pradhan et al found prevalence of ever use of any tobacco product in 19.7% out of 1312 students. Fifty percent used tobacco in
public places and seventy five percent of the consumers purchased tobacco from shops.

In spite of repeated education programme the number of daily tobacco users is increasing and school age is most common age group where one is more prone to get this habit. The large scale education and ban of tobacco products around schools is required. There is need to decrease accessibility of cigarette, totally ban smoking in the public places, and prohibit tobacco advertisement and promotions in Medias and movies.

The shortcoming of present study is small sample size. We evaluated tobacco usage by self administered response, chances of bias in responses exist.

**Conclusion**

Authors found that later age, male gender, nuclear family, monthly income >5000 per month, parental use and private school was predictor and risk factor of tobacco usage among school children.

**Source of Funding**- NIL

**Conflict of Interest**-NIL

**Ethical Clearance**- Taken from: Kalinga Institute of Dental Sciences, Orissa, ethics committee

**References**

Comparative Evaluation of MTA and Biodentine as a Restorative Material In Deciduous Teeth

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Abstract

Introduction: Several medicaments have been used in pulpotomy procedures of primary teeth. The present study was conducted to compare MTA with biodentine as a restorative material in deciduous teeth. Materials & Methods: Specimens were prepared with MTA (group-I) and Biodentine (Group-II) with mold with 10 in each group using stainless steel ring molds. Polyethylene tubes of 1 mm long were filled with MTA and biodentine and placed in flasks containing 10 ml distilled water at 37°C. After 2 h, the flasks were removed from hot air oven, and the water was assessed for pH. Results were tabulated and subjected to statistical analysis.

Results: Each group had 20 teeth sample. In group I, MTA was used and in group II, biodentine material was used. The mean solubility value of MTA at 1st day was 1.55, at 4th day was 1.64, at 12th day was 2.15, at 30th day was 2.57 and at 45th day was 2.60. Similarly in group II at 1st day was 1.89, at 4th day was 2.11, at 12th day was 2.80, at 30th day was 3.61 and at 45th day was 5.34. pH at 2 hours was 9.28 in group I and 9.21 in group II, at 12 hours was 9.26 in group I and 9.39 in group II, at 24 hours was 9.64 in group I and 9.64 in group II, at 7 days was 8.76 in group I and 8.27 in group II, at 28 days was 7.62 in group I and 7.78 in group II. The difference was significant (P< 0.05). Conclusion: Authors found that both materials can be used in deciduous teeth. However, biodentine revealed higher solubility and pH as compared to MTA in deciduous teeth.

Key words: Biodentine, Deciduous, MTA

Introduction

Endodontic treatment of pulpaly involved primary teeth helps to preserve the teeth in non pathological state and to prevent unwanted movement of the neighboring teeth and consequent loss of space in the arch. Moreover, premature tooth loss can lead to malocclusion with aesthetic, phonetic and functional problems that may be transient or permanent teeth. Pulpotomy and pulpectomy are two major treatment modalities for deciduous teeth.

A pulpotomy is performed on a tooth with deep carious lesion, pulp exposure during the operatory process or after a traumatic pulp exposure. Pulpotomy is a treatment procedure that may be performed in healthy radicular pulp tissue with healing potential after surgical removal of the infected or affected coronal pulp. Pulpotomy therapy for the primary dentition can be classified according to treatment objectives as devitalization (mummification, cauterization), preservation (minimal devitalization, non inductive), and regeneration (inductive, reparative) procedure. Pulpectomy is complete removal of dental pulp.

To maintain vitality of primary pulp, to promote healing of the pulp remnants and to maintain the tooth until its natural exfoliation time, numerous pulp medicaments have been used. Among several materials, biodentine and MTA are widely used. Biodentine is composed of powder and liquid. The powder part
includes tricalcium silicate, dicalcium silicate (3 CaO SiO₂ and 2 CaO SiO₂), and calcium carbonate (CaCO₃). Zirconium dioxide (ZrO₂) is added as contrast substance and calcium chloride present as liquid (CaCl₂.2H₂O) for rapid setting time and to improve strength. Mineral trioxide aggregate (MTA) has become more popular, as it has low solubility and good sealing ability.⁵ The present study was conducted to compare MTA with biodentine as a restorative material in deciduous teeth.

**Materials & Method**

This *in vitro* study was performed in the department of Conservative Dentistry and Endodontics. The ethical clearance was obtained from institutional ethical committee before starting the study.

Specimens were prepared with MTA (group-I) and Biodentine (Group-II) with mold with 10 in each group using 20 ring molds of stainless steel having inner diameter of 2.1 cm and a height of 1.6 cm. Mixing of MTA and biodentine was done according to manufacturer’s directions. Following this, the molds were placed inside an incubator cabinet at 37°C.

The mass of dried glass bottles was measured. This procedure was repeated for 4, 12, 30, and 45 days. Polyethylene tubes of 1 mm long were filled with MTA and biodentine and placed in flasks containing 10 ml distilled water at 37°C. After 2 h, the flasks were removed from hot air oven, and the water was assessed for pH. Results were tabulated and subjected to statistical analysis. P value was considered significant at >0.05.

**Results**

**Table I Distribution of teeth**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Group I (MTA)</th>
<th>Group II (Biodentine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Teeth sample</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

Table I shows that each group had 20 teeth sample. In group I, MTA was used and in group II, biodentine material was used.

**Table II Assessment of mean solubility in both groups**

<table>
<thead>
<tr>
<th>Time</th>
<th>Group I</th>
<th>Group II</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day</td>
<td>1.55</td>
<td>1.89</td>
<td></td>
</tr>
<tr>
<td>4 days</td>
<td>1.64</td>
<td>2.11</td>
<td>0.01</td>
</tr>
<tr>
<td>12 days</td>
<td>2.15</td>
<td>2.80</td>
<td></td>
</tr>
<tr>
<td>30 days</td>
<td>2.57</td>
<td>3.61</td>
<td></td>
</tr>
<tr>
<td>45 days</td>
<td>2.60</td>
<td>5.34</td>
<td></td>
</tr>
</tbody>
</table>

Table II, shows that mean solubility value of MTA at 1st day was 1.55, at 4th day was 1.64, at 12th day was 2.15, at 30th day was 2.57 and at 45th day was 2.60. In group II for mean solubility was 1.89 at 1st day, 2.11 at 4th day, at 12th day was 2.80, at 30th day was 3.61 and at 45th day was 5.34. The difference was significant (P< 0.05).

**Table III Assessment of pH in both groups**

<table>
<thead>
<tr>
<th>Time</th>
<th>Group I</th>
<th>Group II</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 hours</td>
<td>9.28</td>
<td>8.98</td>
<td></td>
</tr>
<tr>
<td>12 hours</td>
<td>9.26</td>
<td>9.39</td>
<td>0.01</td>
</tr>
<tr>
<td>24 hours</td>
<td>9.20</td>
<td>9.64</td>
<td></td>
</tr>
<tr>
<td>7 days</td>
<td>8.76</td>
<td>8.27</td>
<td></td>
</tr>
<tr>
<td>28 days</td>
<td>7.62</td>
<td>7.78</td>
<td></td>
</tr>
</tbody>
</table>

Table III Assessment of pH in both groups.
Table III, shows that pH at 2 hours was 9.28 in group I and 8.98 in group II, at 12 hours was 9.26 in group I and 9.39 in group II, at 24 hours was 9.20 in group I and 9.64 in group II, at 7 days was 8.76 in group I and 8.27 in group II, at 28 days was 7.62 in group I and 7.78 in group II. The difference was significant (P< 0.05).

Discussion

MTA and biodentine have become materials of choice in deciduous teeth. Calcium oxide and silicon dioxide are the two main components of MTA. When these raw materials are blended, they produce tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite, and other mineral oxides.6 Radio opacity can be improved with addition of bismuth oxide. Cement hydrates on addition of water, and form silicate hydrate gel. Initially gray MTA was introduced, and to improve esthetic, white MTA was developed later.7

High solubility and prolonged setting time more are the major disadvantages of MTA. Biodentine is consists of the powder component (tricalcium silicate, dicalcium silicate as a second core material, calcium carbonate, oxide as filler, iron oxide shade, and zirconium oxide as a radio-opacifier) and the liquid component (calcium chloride as a setting accelerator and a water reducing agent). There is ample evidence for positive effects of Biodentine on vital pulp cells, for stimulating tertiary dentin formation, and early formation of reparative dentin.8 The present study was conducted to compare MTA with biodentine as a restorative material in deciduous teeth.

In present study, we divided teeth into 2 groups. In group I, MTA was used and in group II, biodentine material was used. Carti et al9 conducted a study in which a total of 25 children with 50 human primary molar teeth aged between 5 and 9 years were selected. The patients were divided into 2 groups. Group I patients received MTA and group II patients received Biodentine. All treated teeth were restored with stainless steel crowns, followed by clinical and radiologic evaluation at 1, 3, 6 and 12 months. There was 96 and 80% of success rate on clinical and radiological evaluation in group I and 96 and 60% in Group II, respectively. There were no statistical significant differences among the groups (P > 0.05). There was decreased success rate on radiographic evaluation in the controls, but it was not statistically significant.

We found that mean solubility value of MTA at 1st day was 1.55, at 4th day was 1.64, at 12th day was 2.15, at 30th day was 2.57 and at 45th day was 2.60. Similarly in group II at 1st day was 1.89, at 4th day was 2.11, at 12th day was 2.80, at 30th day was 3.61 and at 45th day was 5.34. Zaror et al10 performed pulpotomies in primary teeth using MTA and ferric sulphate (FS) and found that radiographic success was 85.71% for the MTA and 83.33% for SF.

We found that pH at 2 hours was 9.28 in group I and 8.98 in group II, at 12 hours was 9.26 in group I and 9.39 in group II, at 24 hours was 9.20 in group I and 9.64 in group II, at 7 days was 8.76 in group I and 8.27 in group II, at 28 days was 7.62 in group I and 7.78 in group II.

Conclusion

Authors found that both materials can be used in deciduous teeth. However, biodentine revealed higher solubility and pH as compared to MTA in deciduous teeth.

Source of Funding- Self

Conflict of Interest - No

Ethical Clearance- Taken from the institutional ethics committee

References


Effects of Circuit Training Program on Quality of Life of Children with Hemophilia

Mandar Malawade¹ Amrutkuvar Rayjade¹, G. Varadharajulu²
¹Associate Professor, ²Professor, Faculty of Physiotherapy, KIMS, Karad

Introduction

Circuit training addresses both the components of exercise i.e. strength and aerobic which can be administered to participants in a playful manner. As the large number of children will have to gather at one place for the intervention, so it will be beneficial for the therapist also to conduct the session by incorporating them into small groups. Very few have shown the effects of circuit training in hemophilia Therefore it becomes necessary to provide an exercise regimen to children which will be interesting to perform in order to improve their general fitness level.

Methodology: Forty nine participants were selected and screened and were allocated into 7 groups. The intervention was given for 4 weeks period, once a day for 7min with 50 sec for exercise and 10 sec to move from one station to the next. Pre and Post parameters were assessed for VAS for fatigue and Subjective Exercise Experience Scale.

Results: Data was analyzed using Graph-Pad InStat software version-trial version 3.03. The statistical measures utilized were mean, standard deviation and test of significance such as Paired ‘t’ test to analyze the data. The results were concluded to be statistically significant with p <0.05 and highly significant with p < 0.01.

Conclusion: The present study concludes that the circuit training is effective in hemophilic children as its multipronged approach improves various body systems.

Key words: Haemophilia, Circuit training, Fatigue, Group Exercises, Physiotherapy

Introduction

The existence of life-long bleeding disorders and their familial occurrence was noted early by Alsaharavius during tenth century.¹ Bleeding disorders are due to either defects in the coagulation mechanism, the blood platelets, vitamin K deficiency or deficiency of clotting factor. Haemophilia is one of the bleeding disorders, has been recognized as a clinical entity since Biblical times.² Hemophilia is also known as ‘the royal disease’ as one famous carrier was Queen Victoria, Queen of England and Ireland, India who transmitted the condition to three of her children.³

Haemophilia has been recognized all over the world, an available estimate range for incidence of haemophilia is from 1 in 20,000 to as high as 1 in 10,000 persons.⁴⁵ It is an X-linked inherited recessive bleeding disorder that is characterized by a deficiency of clotting factor.⁶ Clotting factors are proteins in the blood that controls bleeding.⁷ Haemophilia is traditionally classified as ‘mild’, ‘moderate’, or ‘severe’, depending on the level of clotting factor available in the body compared with that found in the general population.⁸ Patients with severe haemophilia have <1% clotting factor activity,
moderately affected patients have 1-5% and mildly affected patients have 6-40\%\textsuperscript{9}.

Wound bleeding is the characteristic symptoms of all haemophiliacs. It is usually slow and persists for days to weeks. Bleeding into joint may be spontaneous or induced by minor trauma. The earliest joint hemorrhages appear most commonly in knee and ankle, because of the lack of stability of these joints as the toddler assumes an upright posture. Other joints that may be involved are the elbows, hips, wrists, shoulder and small joints of hand and feet.\textsuperscript{10} Prophylactic treatment with the use of coagulation factor is efficient in reducing bleeding episodes in haemophilic children.\textsuperscript{11} Frequent musculoskeletal bleedings ongoing in persons with haemophilia result in limitations and deficiencies of the musculoskeletal system, affecting the performance of daily living activities.\textsuperscript{12} Clinical manifestations may include pain, muscle atrophy, abnormal gait, haemarthrosis, reduced joint range of motion or even the development of degenerative alterations in joint. The damage resulting from haemarthrosis may cause periods of joint immobilization, tendon weakness, stiffness and joint destruction with a higher risk of fractures and osteoporosis.\textsuperscript{3}

Besides pharmacological treatment physiotherapy plays an important role in rehabilitation of haemophiliacs.\textsuperscript{13} Physical fitness is generally defined as ‘the ability to perform daily tasks without fatigue’.\textsuperscript{14} It is an umbrella term incorporating the characteristics of cardiovascular fitness, body compositions, flexibility, muscular strength and endurance.\textsuperscript{15} It is important for the individuals with haemophilia because their disabling condition itself may interfere with their activities like their ability to move efficiently.\textsuperscript{16} Benefits of regular physical exercise for haemophilic patients are numerous and cover various physical and psychosocial aspects. Physical benefits include improvement in range of motion of the joints, muscular strength protecting joint from injury. The psychosocial benefits include higher self-esteem and socialization which leads to a better quality of life.\textsuperscript{18} However physical activity has been linked to improving psychological conditions, such as limiting emotional distress and enhancing self-esteem. Therefore selecting appropriate exercises and fitness activities that are safe for people with hemophilia is essential.\textsuperscript{19}

Circuit training (CT) is a type of exercise which involves exercises to perform in a sequence in a circular manner and designed to improve cardiovascular fitness, muscular strength and endurance. It comprises both forms of exercises i.e. aerobic and resistance or strength training.\textsuperscript{20}

Circuit Training is a fun, cost and time effective way and able to be administered to large groups of individuals at the same time, while still being effective. There is some evidence to suggest that Circuit Training leads to positive changes in body composition, which also improves motor control and fundamental movement abilities. Development of such abilities at a young age may be beneficial in the long term. However, few studies have assessed the effects of Circuit Training on both physical fitness and movement abilities.\textsuperscript{21} Thus, the aim of the present study will be to determine the effectiveness of CT program on Fatigue level and Psychological well-being in children with haemophilia.

**Procedure:**

Forty nine participants were selected for the study. They were randomly allocated into 7 groups. Details about the study and intervention were explained to the participants and written informed consent was obtained. Demographic details of each participant were noted including clotting factor level. Participants were assessed pre and post data for Visual Analogue Scale for fatigue and Subjective Exercise Experience Scale.

The intervention was given once a day for 4 weeks period, once a day, for 7min with 50 sec for exercise and 10 sec to move from one station to the next in the form of Circuit Training. A demonstration was given to the participants to make them understand about how to perform the exercises and how to move in the Circuit. Exercises were done with music for having effective and enjoyable. At a time only one group was given Circuit Training followed by other in a circular sequence. Each participant in the circle performed respective exercise recommended to them at that particular station.

**Exercises for respective joints were the followings:**\textsuperscript{22}

**Exercise for Shoulder joint:** Keep both the hands on shoulders of the same side. Move the shoulders in
outward direction in a circular way simultaneously.

**Exercise for Elbow joint**: Stand facing the wall. Place hands flat on wall with arms straight. Lean body towards wall allowing elbow to bend. Return to upright position by pushing with arms and straightening the elbow.

**Exercise for wrist joint**: Place palm of hands together with finger straight (in a prayer position) while keeping the hands pressed flat together move both the wrists in extension alternately.

**Exercise for Hip joint**: Stand by facing the wall. Place both the hands on the wall for support. Extend the hip by keeping the knee straight. Perform the same for both the hips alternately.

**Exercise for Knee joint**: Stand with the weight evenly distributed on both feet. Squat down partway keeping weight distributed evenly on both feet. Do not bend knees far enough to cause pain. Hold for several seconds. Return to upright position.

**Exercise for Ankle joint**: Stand with both legs adequately wide apart. Place both palms of hands on wall for support. Do the dorsi-flexion and plantar-flexion alternately.

**Exercise for Trunk**: Keep both the hands on low back for the support and gently extend as much as possible. Then come back to upright position.

**Data Analysis**

Data was analyzed using Graph-PadInstat software trial version 3.03. It was entered into an excel spread sheet, tabulated and subjected to statistical analysis. The statistical measures utilized were mean, standard deviation (SD) and test of significance such as Paired 't’ test to analyze the data. Paired ‘t’ test was used to compare the differences of scores on pre and post intervention within a single group. The results were concluded to be statistically significant with p < 0.05 and highly significant with p < 0.01.

**Fatigue Score**:

The rates of perceived fatigue were assessed by VAS. The rating of fatigue scores were recorded on the scale. Paired ‘t’ test was used to compare score within the group. The pre-intervention average score of fatigue score in participants was 1.40 and post-intervention was 1.87. There was highly significant increment in fatigue score for the group after intervention.

**Table 1: Comparison of pre and post intervention score of Fatigue**

<table>
<thead>
<tr>
<th>Fatigue Score</th>
<th>Mean ± SD</th>
<th>Mean ± SD</th>
<th>Mean difference</th>
<th>‘t’ value</th>
<th>P value</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.40 ± 1.17</td>
<td>1.87 ± 1.50</td>
<td>0.673±0.74</td>
<td>3.683</td>
<td>0.006</td>
<td>Highly significant</td>
</tr>
</tbody>
</table>

**Subjective Exercise Experience Scale (SEES)**: Paired ‘t’ test was used to compare score within the group. The pre-intervention average score of Positive wellbeing in participants was 14.32 and post-intervention was 16.97. There was highly significant increment in Positive wellbeing score for the group after intervention.

The pre-intervention average score of Psychological Distress in participants was 7.14 and post-intervention was 4.83. There was highly significant reduction in Psychological distress for the group after intervention.

The pre-intervention average score of fatigue was 5.51 and post-intervention was 5.10. There was highly increment in positive wellbeing component as well as significant reduction Psychological distress component of SEES after intervention, whereas Fatigue component found to be significant.
Table 2: Comparison of pre and post intervention scores of SEES components

<table>
<thead>
<tr>
<th>SEES Components</th>
<th>PRE Mean + SD</th>
<th>POST Mean + SD</th>
<th>Mean Difference</th>
<th>‘t’ value</th>
<th>‘p’ value</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive well being</td>
<td>14.32 + 2.42</td>
<td>16.97 + 2.17</td>
<td>2.65±0.96</td>
<td>19.15</td>
<td>0.001</td>
<td>Highly significant</td>
</tr>
<tr>
<td>Psychological Distress</td>
<td>7.14 + 1.39</td>
<td>4.83 + 1.28</td>
<td>2.30±0.821</td>
<td>19.64</td>
<td>0.001</td>
<td>Highly significant</td>
</tr>
<tr>
<td>Fatigue</td>
<td>5.51 + 1.17</td>
<td>5.10 + 1.18</td>
<td>0.93±0.59</td>
<td>2.74</td>
<td>0.08</td>
<td>Significant</td>
</tr>
</tbody>
</table>

Discussion

The study was done to evaluate the effects of circuit training in Haemophilic Children. In children with Haemophilia reduced muscle strength and cardiovascular endurance are main contributing factors to have poor fitness level and reduced psychological wellbeing compared to typically developing peer which results because of fear of getting bleed.

Circuit Training showed good improvement in Positive wellbeing, reduced psychological distress and fatigue to some extent. Circuit training being multipronged in its approach addresses the improvement in various systems of body and even psychological aspects. CT program was specifically designed to provide variation and stimulation in order to maximally engage children and to ensure adherence and enjoyment, whilst achieving positive changes in health and fitness outcome measures. The effects seen in this study were due to gradual effects of exercise which imposes demands on the different systems of the body such as enhancement of oxygen delivery systems, expanded plasma volume, maximum minute ventilation and increased cellular enzyme activity.23, 24

Most of the haemophilic children are sedentary and having reduced physical activity. Therefore fatigue had set in early. In the present study there was significant difference fatigue score.

A pilot study was done to determine the effect of an acute bout of exercise on the subjective exercise experience of Vietnam Veterans, and to determine which exercise intervention results in the most positive acute outcome. Specifically, this study included the use of a combination of traditional exercise modes and innovative exercise. Overall this pilot study has demonstrated that an acute bout of exercise elicits improved positive wellbeing and reduced psychological distress, and therefore warrants further research with a larger cohort to determine which exercise intervention results in the most beneficial subjective experience. The identification of specific exercise interventions that elicit acute positive exercise experiences has important implications for long-term exercise adherence in at-risk populations.

In the present study there were significant differences in the components of Subjective Exercise Experience Scale (SEES) with increase in positive wellbeing. Psychological distress showed reduction in score after intervention, whereas fatigue component also showed significant difference.
A study has proved that a school-based CT program had beneficial effects on a range of fitness measures. These movement skills are helpful in ensuring good general posture, and effective movement in a variety of physical activities, sports and game play. For these reasons, and given its low cost and suitability for large groups, CT maybe a useful exercise method to adopt in schools. This is in accordance with the research studies, CT program on the health, fitness, physical activity levels, and movement competency in young children.25, 26

An overview of literature review concluded that aerobic and resistant training intervention elicits some benefits for physical fitness and blood coagulation mechanisms, suggesting the application of physical training as a non-pharmacological treatment in association with conventional treatment. Adequate and periodized resistance training considering the disease severity, accompanied by physical education professionals could improve muscle strength, balance and proprioception. Aerobic training could reduces the risks of obesity and several metabolic and cardiovascular diseases. Another review article concluded important evidence on the recommendation of physical exercise and sport in haemophilia, the consensus on its suitability for the physical and social wellbeing of the patients and the need to increase scientific works in this respect.27

Researchers analyzed the current state of management of haemophilia and to suggest a comprehensive concept of rational and effective rehabilitation of children with haemophilia. The study concluded that current rehabilitation care is of good quality and necessary for effective lifelong therapy of haemophilia.28

Thus many researchers have proved that for achieving general fitness and maintaining psychological wellbeing in haemophilic children, exercise regimen and physical activities contribute major role which occurs due to physiological changes in various systems of body in order to prepare the body to control the new imposed demands while performing the exercise and physical activities.

**Conclusion**

The present study concludes that the circuit training is effective in haemophilic children as its multipronged approach improves various body systems.

**Ethical Clearance**- Taken from Krishna Institute of Medical Science Ethical committee

**Source of Funding**- Self

**Conflict of Interest** - Nil

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Assessment of Serum Levels of Salusin α and Salusin β in Cardiovascular Disease Patients Undergoing Transcatheter Therapy

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Abstract

Cardiovascular disease (CVD) covers some disorders, such as the diseases of the cardiac muscles and the vascular system supplying the heart, brain and extra vital organs. CVD morbidity and mortality is mainly due to coronary heart disease and cerebro-vascular diseases. The aims of the present is to measure the levels of Salusin α, Salusin β, lipid profile, fasting blood sugar, insulin, insulin resistance and HOMA IR in the sera of cardiovascular patients who are subject to catheterization and to compare these levels with the ones of the healthy group and their correlation with Salusins. The results have made use of the appropriate statistical methods.

The results of the research have shown that there is a significant increase in the salusin-β in CVD patients compared with that of the healthy group but Salusin α (p›0.001) witnessed a significantly lower level. The present study has demonstrated that VLDL.C, HDL.C, TG.C and Salusin-β are in a significant correlation with Salusin α. Also, Age, BMI, HOMA-β, Insulin, HOMA-IR, FBG, CHO and LDL.C have no significant correlation with Salusin α. The results have shown that BMI and Salusin α levels are significantly correlated with salusin-β. In addition, Age, HOMA-β, Insulin, HOMA-IR, FBG, CHO, LDL.C, VLDL.C, HDL.C and TG.C have no significant correlation with Salusin-β. BMI and HDL.C have shown a positive correlation with Salusin α. Moreover, Age, Insulin, HOMA-IR, HOMA-β, FBG, CHO, LDL.C, VLDL.C and TG.C have shown a positive correlation with Salusin-β.

Key words: Cardiovascular disease, Salusin α, Salusin β.

Introduction

Cardiovascular disease (CVD) covers disorders like the cardiac muscles and the vascular system supplying the heart, brain and extra vital organs (1). CVD morbidity and mortality is mainly due to coronary heart disease and cerebro-vascular diseases (2). CVD is as a rule connected with atherosclerosis; an inflammatory disease characterized via the accumulation of lipids and fibrous elements in relation to medium arteries (3).

Salusins are taken as a category of bioactive peptides discovered through bioinformatics analyses of a complete length CDNA library. Recently, two associated peptides of 28 and 20 amino acids are renowned and characterized; they specified Salusin-α and Salusin-β. These peptides are believed to be biosynthesized of pre-prosalusin; an alternative-splicing production of the torsion dystonia–related gene (TOR2A), subsequent to frame shift reading and digestion at dibasic amino acids (4).

Salusins are synthesized ubiquitously in human tissues, counting the vasculature, central nervous system and the kidney. Salusin-α is present inside human plasma and urine (5). Salusin-β quickly induces hypotension, bradycardia, and cardiac dysfunction during a cholinergic mechanism (6). Salusin- β also stimulates human macrophage foam cell formation (7), proliferation of vascular smooth muscle cells and fibroblasts (4) and cardiomyocyte growth and anti-apoptosis (8,9).

Material and Method

A case control study is designed for a total of 60 subjects (44 males and 16 females, aged between 35 to
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65 years) who consecutively registered in this study. The subjects are 30 Iraqi patients with cardio vascular disease (CVD). They have participated in the current study (8 female and 22 males). These patients are registered as CVD patients in the “open heart Unit” at “AL-Sader Teaching Hospital” in Najaf, Iraq. The patients' serum in the pre - and post-cardiac catheterization is collected and compared with that of the control group. Thirty healthy adults are selected as the control group. The range of their ages is analogous to that of the patients (35-65) years. Subjects who suffer from the apparent diabetes mellitus, acute infections, chronic liver diseases, renal disorders, cancers and patients with surgical procedures in the last 3 months, and nonsmoker are all excluded.

Five milliliters of venous blood after 12 hours fasting are drawn from the CVD patients and the healthy group during (8:30-10 A.M) from antecubital venipuncture using G 23 needles.

Hypertension is diagnosed as a systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg. The BMI is calculated as the ratio of weight (Kg) to height squared (m²), by unit kg/m². Fasting analysis of serum glucose, lipid profile (CHO, TG.C, LDLC, and HDL.C) levels are measured by colorimetric method for the quantitative in vitro diagnostic measurement using kit (BIOLABO (France)). The Salusin α and Salusin-β are also measured using the Competitive-ELISA principle (Elabscience (USA)). The concentrations of Insulin serum are determined by ELISA kits (Calbiotech (USA)). Insulin resistance index (Homeostatic model assessment-insulin resistance,HOMA-IR) is estimated as follows: HOMA- IR = [glucose (in mg/dL) * insulin (μU/ml)] / 405. HOMA-α = (360 × Insulin/(Glucose-63)) % (10). The results are subjected to statistical analysis and are analyzed using Microsoft Excel 2013 and SPSS-20 (statistical package for social science-version 20). The results are expressed as numbers and as mean ± SD (Standard deviation). The significance of difference is assessed using paired t-test for two dependent means. The correlation of parameters is determined using Pearson’s correlation coefficient, taking p≤0.05 as the lowest limit of significance (11).

Bio-statistical Analysis

The results are subjected to statistical analysis and are analyzed using Microsoft Excel 2013 and SPSS-20 (statistical package for social science-version 20). The results are expressed as numbers and as mean ± SD (Standard deviation). The significance of difference is assessed using paired t-test for two dependent means. The correlation of parameters is determined using Pearson’s correlation coefficient, taking p≤0.05 as the lowest limit of significance (11). The one-way ANOVA (Analysis of variance) and Fishers Least Significant Difference (LSD) are applied to compare the differences among the studied groups.

Results and Discussion

Clinical characteristics of the Studied Groups

The patients with cardiovascular diseases have shown a significantly higher level of fasting blood sugar (by definition, (p<0.001), higher level of insulin(p<0.001), respectively), higher level of HOMA IR(p<0.001), Lipid profile (p<0.001) and Salusin-β (p<0.001) in CVD patients (Group2), compared with the healthy group (Group1). Additionally, lower Salusinα and HOMA-β (p<0.001) are also seen, table (1). Table (1) shows significantly higher levels in the Insulin, FBG and HOMA IR (p<0.001), except HOMA-β which records a non significant level in pre-catheterization (Group2), compared with post-catheterization (Group3). No significant difference is registered regarding salusin-β and insulin (p<0.001)

But there is a significant increase in FBG, HOMA IR, HOMA-β,Lipid profile, Salusinα, when the control (Group1) is compared with post-Catheterization (Group3).

The demographics and laboratory data of the Salusin α and Salusin β groups are summarised in tables (2) and (3). The levels of VLDL.C, HDL.C, TG.C and Salusin β are significantly higher with Salusin α for all (p<0.05)( p =0.036) (p=0.022) (p=0.035), (p=0.048), respectively.

There is no significance correlation of Age, BMI, HOMA-β, Insulin, HOMA-IR, FBG, CHO and LDL with Salusin α (p›0.001), as in table (2).

BMI and Salusin α levels are significantly elevated with Salusin-β (p<0.05) (p=0.033) (p= 0.048), respectively. Moreover, Age, HOMA-β, Insulin, HOMA-IR, FBG, CHO, LDL.C, VLDL.C, HDL.C and TG.C have no significant correlation with Salusin-β(p›0.001), as in table (3).

This study has found that HDLC is positively correlated with Salusin α. In addition, Age, BMI, Insulin, HOMA-β, HOMA-IR, FBG, CHO, LDL.C, VLDL.C, TG.C and Salusin-β showed a negative correlation with Salusin α, as in table (2).
Furthermore, HDL.C and Salusin α showed a negative correlation with salusin-β. Also, Age, BMI, Insulin, HOMA-IR, HOMA-β, FBG ,CHO, LDL.C, VLDL.C and TG.C showed a positive correlation with Salusin-β., as seen in table (3).

CVD is as a rule connected with atherosclerosis (3). Atherosclerosis is a chronic vascular disease, in which the arteries are thicken and lose their flexibility as a result of cholesterol sedimentation in the artery wall. In the early stages of the disease, cholesterol accumulates in arterial macrophages, alter them to lipid-loaded bubbles cells. Wide atherosclerosis narrows the artery lumen to reduce blood flow, and the can enhance the complete blockage of the artery (12; 13). Atherosclerosis occurs due to a variety of reasons, the most significant of which is the deposition of large amounts of cholesterol and calcium in the blood. It may happen because of obesity overcharged as a result of absence of exercise. Additionally, the increased blood pressure raises the risk of developing atherosclerosis (14; 15).

### Table (1) Biochemical characteristics of CVD patients and healthy as control group

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group1 Mean±SD</th>
<th>Pre-catheter group2 Mean±SD</th>
<th>Post-catheter group3 Mean±SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>28.01±1.34</td>
<td>29.11±1.27</td>
<td>29.11±1.27</td>
<td>a) NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>b) NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>c) NS</td>
</tr>
<tr>
<td>BMI</td>
<td>26.32 ± 3.329</td>
<td>31.46 ±5.010</td>
<td>31.46 ±5.010</td>
<td>a) 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>b) 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>c) 0.001</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>122.6±1</td>
<td>145±2</td>
<td>139.5±2</td>
<td>a) &lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>b) &lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>c) NS</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>70.7±2</td>
<td>76.3±2</td>
<td>71.2±2</td>
<td>a) NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>b) NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>c) NS</td>
</tr>
<tr>
<td>FBG (mg/dl)</td>
<td>99.29 ± 8.52</td>
<td>166.62±35.24</td>
<td>143.87±36.17</td>
<td>a) 0.000**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>b) 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>c) 0.019</td>
</tr>
<tr>
<td>Insulin(μu/ml)</td>
<td>10.67±3.30</td>
<td>19.43±10.85</td>
<td>8.94±4.05</td>
<td>a)0.000**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>b) NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>c) 0.000**</td>
</tr>
<tr>
<td>HOMA IR</td>
<td>2.59 ±0.82</td>
<td>8.03 ± 4.85</td>
<td>3.33 ± 2.21</td>
<td>a)0.000**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>b) 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>c) 0.000**</td>
</tr>
<tr>
<td>HOMA-β</td>
<td>111.42±43.88</td>
<td>145.58±369.51</td>
<td>45.42±24.85</td>
<td>a)0.617NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>b) 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>c) 0.151 NS</td>
</tr>
<tr>
<td>HDL.C</td>
<td>55.61±6.33</td>
<td>37.43 ±8.41</td>
<td>39.07±6.97</td>
<td>a)0.000**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>b) 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>c) 0.419 NS</td>
</tr>
<tr>
<td>VLDL.C</td>
<td>20.23±3.31</td>
<td>51.48±16.86</td>
<td>39.79±13.28</td>
<td>a)0.000**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>b) 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>c) 0.005**</td>
</tr>
<tr>
<td>LDL.C</td>
<td>86.00±14.95</td>
<td>185.48±45.20</td>
<td>165.93±45.37</td>
<td>a)0.000**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>b) 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>c) 0.106 NS</td>
</tr>
</tbody>
</table>
Cont... Table (1) Biochemical characteristics of CVD patients and healthy as control group

<table>
<thead>
<tr>
<th>Variables</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.000</td>
<td>1.000</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.304</td>
<td>0.109</td>
</tr>
<tr>
<td>FBG</td>
<td>-0.164</td>
<td>0.394</td>
</tr>
<tr>
<td>Insulin(µIU/ml)</td>
<td>-0.179</td>
<td>0.353</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>-0.227</td>
<td>0.237</td>
</tr>
<tr>
<td>HOMA-β</td>
<td>-0.077</td>
<td>0.693</td>
</tr>
<tr>
<td>CHO</td>
<td>-0.194</td>
<td>0.314</td>
</tr>
<tr>
<td>LDL.C</td>
<td>-0.119</td>
<td>0.538</td>
</tr>
<tr>
<td>VLDL.C</td>
<td>-0.392*</td>
<td>0.036</td>
</tr>
<tr>
<td>HDL.C</td>
<td>0.425</td>
<td>0.022</td>
</tr>
<tr>
<td>TG.C</td>
<td>-0.394*</td>
<td>0.035</td>
</tr>
<tr>
<td>Salusinβ(pg/ml)</td>
<td>0.371*</td>
<td>0.048</td>
</tr>
</tbody>
</table>

P- Value ≤ 0.05 = significant, r : Pearson correlation

a) Significant difference between values in Group(1) and Group (2), b) Significant difference between values in Group (3) and Group (1), c) Significant difference between values in Group (3) and Group (2), **=significant differences at 1%, NS =non-significant at the 0.05 level,

FBG: fasting blood glucose, HOMA-IR: Homoeostasis model assessment-insulin resistance.Salusin-α has a mild hypotensive effect (4) and suppresses human foam cell formation via the down-regulation of acyl-CoA : cholesterol acyltransferase-1 (ACAT-1), which stores cholesterol ester changed from free cholesterol in macrophages. In previous studies, Serum salusin-α levels are significantly decreased in acute coronary syndrome (ACS) patients as compared with healthy people and are less in accordance with the severity of coronary atherosclerotic lesions amongst ACS patients. In coronary atherosclerotic lesions of ACS patients, the level of expression of Salusin-α is lower than that of Salusin- β (7).

Salusins essentially affect the cardiovascular system (6;7). Salusin-β is the most hypotensive peptide, its infusion is rapid and profoundly decreases blood pressure and heart rate. Moreover, it is demonstrated to cause cardiac dysfunction through a cholinergic mechanism in rats (6;8). It may have significant roles in myocardial growth and hypertrophy. Salusin-α and salusin-β show converse actions on atherosclerosis due to their opposite regulatory effects on acyl-coenzyme A: cholesterol acyltransferases-1 (ACAT-1). Both of the formation of macrophage foam cells and the enlargement of atherosclerosis are suppressed via Salusin-α. Serum Salusin-α levels are also reported to be significantly lower in patients with coronary artery disease and hypertensive patients where their Salusin-α level is inversely associated with carotid atherosclerosis (7;16).
### Table(3) The relevance of Salusin β with concentrations of biochemical parameters in the patients group

<table>
<thead>
<tr>
<th>Variables</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.182</td>
<td>0.345</td>
</tr>
<tr>
<td>BMI</td>
<td>0.397*</td>
<td>0.033</td>
</tr>
<tr>
<td>FBG</td>
<td>0.122</td>
<td>0.527</td>
</tr>
<tr>
<td>Insulin(µIU/ml)</td>
<td>0.127</td>
<td>0.511</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.102</td>
<td>0.597</td>
</tr>
<tr>
<td>HOMA-β</td>
<td>0.225</td>
<td>0.240</td>
</tr>
<tr>
<td>CHO</td>
<td>0.038</td>
<td>0.844</td>
</tr>
<tr>
<td>LDL.C</td>
<td>0.118</td>
<td>0.542</td>
</tr>
<tr>
<td>VLDL.C</td>
<td>0.150</td>
<td>0.438</td>
</tr>
<tr>
<td>HDL.C</td>
<td>-0.125</td>
<td>0.517</td>
</tr>
<tr>
<td>TG.C</td>
<td>0.149</td>
<td>0.441</td>
</tr>
<tr>
<td>Salusin α (pg/ml)</td>
<td>-0.371*</td>
<td>0.048</td>
</tr>
</tbody>
</table>

P- Value ≤ 0.05 = significant, r : Pearson correlation

Angiotensin II is associated with the genesis of arterial hypertension and cardiovascular remodeling (17, 18). Renin-angiotensin system intervention in hypertensive patients has shown lower morbidity and mortality (19; 20). Salusin-β gene silence has normalized the increased circulating Ang II levels in addition to the local Ang II contents in both myocardium and mesenteric artery in spontaneously hypertensive rats (SHR). Additionally, the up-regulation of AT receptors within myocardium and mesenteric artery in SHR (4) are inhibited through the knockdown of Salusin-β.

The inhibitory effect of Salusin-β on the activation of angiotensin system may partially contribute to the attenuation of hypertension and cardiovascular remodeling. It is well known that increased oxidative stress is associated with endothelial dysfunction, apoptosis, hypertrophy, inflammation, fibrosis and cell migration relative to vascular remodeling of hypertension (21; 22).

### Conclusion

In conclusion, our results suggest that Salusin-α and Salusin-β prove contrasting effects on atherosclerosis and that Salusin-α and Salusin-β possess anti-atherogenesis and proatherogenesis, respectively.

The current study has concluded that in patients with CVD, Salusin β levels have recorded a significant increase in the serum of CVD.

Further, Lipid Profile changes are directed by the age of patients in CVD.

Salusinα increase in serum of post catheterization and Salusin β decrease in serum of post catheterization.

In order to improve diagnosis and treatment of CAD, the research community needs to understood how the immune response is analogous and how it differs in men and women with atherosclerosis.

**Acknowledgement:** I would like to thank my Supervisor Dr. Hanaa Addai Ali for her helpful comments and assistance with this study.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** Self-funding

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Effect of Multi-Wall Carbon Nanotubes on the Microhardness of the Tooth Enamel

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Abstract

Background: The object of this study was to investigate the effect of addition of Multi-Wall Carbon Nano Tubes (MWCNTs) in different concentration (0.001g/20ml, 0.005g/20ml, 0.01g/20ml and 0.02g/20ml) in (dimethyl sulphoxide) to tooth enamel. It is intended to evaluate enamel hardness in (Kg .m-2) before and after the addition of (MWCNTs).

Materials and Method: Thirty specimens were prepared for this study, to measure the hardness of the enamel.

Results: The results enamel hardness after MWCNTs application With DMSO showed a significant increase (P<0.01) between groups of 0.001g/20ml, 0.005g/20ml, 0.01g/20ml and 0.02g/20ml concentration compared with control group. Where the highest mean value obtained was in 0.02g/20ml concentration which had a mean value of 527.18 ± 2.904 Kg .m-2, while the lowest mean value was for control group which had a mean value of 334.87± 2.904 Kg .m-2. Polished enamel surface groups showed a significant increase 452.59 ± 1.84 Kg .m-2 (P<0.01) in hardness when compared with unpolished enamel surface groups 430.16 ± 1.84 Kg .m-2 (P<0.01).

Conclusion: The highest mean value obtained was in 0.02g/20ml concentration in the hardness suspended in DMSO. The results showed a significant increase in the hardness for polished enamel surface samples compared with unpolished enamel surface samples in DMSO.

Key words: Multi-Wall Carbon NanoTubes (MWCNTs), enamel hardness

Introduction

Enamel, the outer hard tissue layer of tooth crowns, is a composite material that comparable to other biological tissues like bone or dentin exhibits a unique and complex hierarchical structure (1). The bulk of human teeth consists of two main mineralized tissues, collagen-rich dentine and highly mineralised enamel. They join forming a complex and mechanically durable dentine–enamel junction (DEJ) that contributes to the lifelong success of the tooth structure under thermo-mechanical loadings encountered in the oral cavity under the conditions such as mastication, chemically active environment and thermal shock (2, 3).

Enamel is the hardest tissue in the human body and is considered a nanostructured biocomposite in which its mineral phase predominates (95-96 wt. %) (4). In this mineral portion, large hexagonal carbonated hydroxyapatite crystals are tightly packed creating prisms with a keyhole-like structure of about 5 µm in diameter (5). Prisms are aligned and run approximately perpendicular from the dentin-enamel junction to the tooth surface (3). Each prism is separated from each other by a nanometer-thin layer of a protein-based organic matrix (6). The term “Nano” is derived from the Greek word “dwarf”. More simply speaking, one nanometer is one-billionth or 10-9 of a meter (7-9). Nanotechnology can be classified in terms of application in three broad and extensively overlapping categories (10):
\begin{itemize}
\item Nanoelectronics
\item Nanomaterials/particles
\item Nano-biotechnology
\end{itemize}

Carbon nanotubes (CNT) are a new crystalline form of carbon. Wound in a hexagonal network of carbon atoms constituting a graphene nanofoil, these hollow cylinders can have diameters as small as 0.7 nm with lengths that can range from a few micrometres, and reach several millimeters in length (11). Each end can be opened or closed by a fullerene half molecule. These nanotubes can have a single layer (SWCNT for single walled carbon nanotube) or several layers (MWCNTs for multi walled carbon nanotube) of coaxial cylinders of increasing diameters in a common axis. Multilayer carbon nanotubes can reach diameters of 100 nm (12).

Enamel surface microhardness refers to a tooth’s resistance to scratching, abrasion, and indentation. A substantial number of mineral ions can be removed from hydroxyapatite latticework without destroying its structural integrity; however, such demineralized enamel transmits hot, cold, pressure and pain much more readily than normal enamel. Microhardness tests are commonly used to study the physical properties of materials, and they are widely used to measure the hardness of teeth (13, 14). Knoop (KHN) and Vicker (VHN) hardnesses have reported approximately the same value (15). The average hardness value for enamel and dentin is in the range from 270 to 350 Knoop microhardness or from 250 to 360 Vickers microhardness and from 50 to 70 Knoop microhardness respectively (16).

**Functionalisation of Commercial Carbon Nano Tubes** (17). One gram MWCNTs was transferred into a mixture of nitric acid (10 cm3) and sulphuric acid (30cm3). This mixture was then heated at 50 °C for 24 hrs after which the MWCNTs were filtered off using Nylon filter paper (pore size 0.45 micrometer). This was followed by subsequent washing with distilled water until the pH was almost neutral. The MWCNTs were then dried under vacuum at room temperature. Then dried in furnace oven for 2hr.In the third step 0.02 g from MWCNTs were put in 20 ml DMSO. The MWCNTs is mixed with DMSO. The whole solution is transferred into sonicator. Twenty four sample in DMSO in different concentration for micro hardness test. The samples should be kept in a water. These samples were shaken in the vibrator for limited period extend 10min for 3 time in 6 continuous days.

**Grouping of Samples**

The group contains 30 samples to measure the hardness of the enamel. Samples were collected from healthy teeth of patients attending a dental teaching hospital at the University of Baghdad college of Dentistry, also Thi-Qar specialized dental center in department of Orthodontics of the ages ranging between 15 - 24 years. The first selection criterion for the sample was tooth quality. Only teeth with no visible defects were selected, not taking into account any damage at the micro structural level. They were without any caries, no attrition or erosion. The patients were non-smokers and do not consume alcoholic beverages. The second selection criterion was that the teeth belonged to mandibular first premolar.

**The Hardness of a material**

The hardness of a material its resistance to penetration under a localized pressure or resistance to abrasion. The baseline of the hardness of base lines was measured through the use of Micro -Vickers Hardness Testing Machine (CV-400 DM, Europe) (Figure1), with a load of 500 g and 1000 g, in 5 seconds.

**Principle of Hardness Determination**

The micro hardness test involves a microscopic and static method, of which the results are mostly expressed in terms of Vickers and Knoop hardness numbers. The micro hardness tester is provided with an optical magnifying system. The hardness is determined by penetrating a diamond pyramid indenter under a known test force into the surface of test piece and then measuring the diagonal of the indentation left on the surface after removal of the test force.

The hardness number is calculated upon the below equations.

\[
HV = \frac{1854}{4F/d}
\]

Vickers Test

HV: Vickers hardness number, in kg f. mm-2, F : Test force, in kg f, d :Diagonal length of the indentation, in mm2.

**Sample Preparation to Measure The Hardness**
The total number of samples were 30 samples to measure the hardness of the enamel, and divided the group to subgroups upon the following design.

**Control group**: (3 unpolished enamel surface and 3 polished enamel surface samples).

**Group (0.001g/20ml)**: (3 unpolished enamel surface and 3 polished enamel surface samples)

**Group (0.005g/20ml)**: (3 unpolished enamel surface and 3 polished enamel surface samples)

**Group (0.01g/20ml)**: (3 unpolished enamel surface samples)

**Group (0.02g/20ml)**: (3 unpolished enamel surface samples)

Groups (0.001g/20ml, 0.005g/20ml, 0.01g/20ml, 0.02g/20ml) treated with MWCNTs, the special area on the middle third of the labial enamel surface were chosen 5mm away about the cusp tip and whit a line representing axis (X) for all teeth. All have samples examined for the micro hardness.

**Statistical Analysis**

Statistical analysis was done using the software SPSS version 17.0; the results were expressed as mean ± standard deviations (mean ± SE). One way ANOVA was used to compare parameters in different studied groups. P-values (P < 0.01) were considered statistically significant.

**Results**

Statistical Analysis of the results was used to evaluate, enamel hardness in (Kg.m-2) after MWCNTs application with DMSO in different concentration treatment.

Enamel Hardness Test

Control group comport with groups dealing with MWCNTs application with DMSO in different concentration treatment and different surfaces treatment.

MWCNTs application with DMSO in different concentration treatment and different surfaces treatment.

Table (1) showed that the highest mean value found in 0.02g/20ml concentration which has a mean value of 527.18 ± 2.904 Kg.m-2, while the lowest mean value was for the control group which has a mean value of 334.87 ± 2.904 Kg.m-2. The results of LSD test showed a statistically significant differences between groups in different concentration treatment as in the figure (2). And also table (1) showed that the highest mean value represent in polished enamel surfaces which has a mean value of 452.59 ± 1.84 Kg.m-2, while the lowest mean value was for in unpolished enamel surfaces which has a mean value of 430.16 ± 1.84 Kg.m-2. The results of LSD test showed a statistically significant differences between groups in different surfaces treatment as in the figure (3).

Table (1) showed that the highest mean value present a concentration of 0.02g/20ml in polished surfaces which has a mean value of 547.07 Kg.m-2, while the lowest mean value was for the control group in unpolished surfaces which has a mean value of 330.30 Kg.m-2 and the results of LSD test showed a statistically significant differences between groups in different concentration treatment and different surfaces treatment.

**Discussion**

**Micro Hardness of Dental Enamel**

Based on the findings of the current study, the average value of Vickers enamel microhardness was 334.87 ± 2.91, which is similar to the findings of Panich and Poothong (18), enamel hardness depends on different factors such as degree of enamel mineralization, enamel prisms and enamel tufts variations in different areas of enamel, presence or absence of any structural defects in the enamel, type of the teeth (whether it is anterior or posterior), and procedures for preparing the samples to perform the hardness test (13). Other factors influencing enamel hardness are the bio environmental factors, fluoridation of the drinking water, age of the teeth, and different eating habits in different societies (19).

**Enamel Hardness After MWCNTs Application With DMSO**

The results of the hardness are reported in Table (1) and figure (2) from which it is observed that the highest mean value obtained was in concentration 0.02g/20ml which had a mean value of 527.18 ± 2.904 Kg.m-2, while the lowest mean value was for control group which had a mean value of 334.87± 2.904 Kg.m-2. The results showed a significant increase in hardness between groups in concentration 0.001g/20ml, 0.005 g/20ml, 0.01 g/20ml and, 0.02 g/20ml compared with control group. Peter Atkin’s and Julio de paula (20)
it’s well known that CNTs are thin cylinders of carbon atoms that are mechanically strong. At this time, the increase in hardness due to the increase in concentration of MWCNTs figure (4), the tremendous surface area of CNTs up to 200 m2.g-1 leads to formation of clusters due to Van Der Waals forces. Clustering and non-uniform dispersion of CNTs will lead to inhomogeneous property distribution in the structural component (21). The results of the hardness are reported in Table (1) and figure (3) from which it is observed that the highest mean value of polished enamel surface which had a mean value of 452.59 ± 1.84Kg .m-2 ,while the lowest mean value of unpolished enamel surface which had a mean value of 430.16 ± 1.84 Kg .m-2 . The results showed a significant increase in the hardness for polished samples compared with unpolished samples. Because the surface area for polished samples more than unpolished samples. Since the of the polished surface leads to increases the surface area of dental enamel attached to them MWCNTs material, which leads to increased the permeability of the materials to the inside enamel rods ,this leads to increased material inside dental enamel, gives an increase in the enamel hardness (22).

Table (1): Descriptive data of enamel hardness in (Kg .m-2) after MWCNTs application with DMSO of the relationship between different concentration treatment and different surfaces treatment

<table>
<thead>
<tr>
<th>Concentration g.mol-1</th>
<th>Subjects no.</th>
<th>Unpolished</th>
<th>Polished</th>
<th>Mean ± S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>330.30 g</td>
<td>339.43 g</td>
<td>334.87 ± 2.904 e</td>
</tr>
<tr>
<td>0.001/20</td>
<td>6</td>
<td>396.27 g</td>
<td>421.73 e</td>
<td>409.00 ± 2.904 d</td>
</tr>
<tr>
<td>0.005/20</td>
<td>6</td>
<td>437.67 e</td>
<td>465.13 d</td>
<td>451.40 ± 2.904 c</td>
</tr>
<tr>
<td>0.01/20</td>
<td>6</td>
<td>479.27 cd</td>
<td>489.60 c</td>
<td>484.43 ± 2.904 b</td>
</tr>
<tr>
<td>0.02/20</td>
<td>6</td>
<td>507.30 b</td>
<td>547.07 a</td>
<td>527.18 ± 2.904 a</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>430.16 ± 1.84 b</td>
<td>452.59 ± 1.84 a</td>
<td>LSD0.01 (concentration) = 9.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LSD 0.01 (surfaces ) = 6.25</td>
</tr>
</tbody>
</table>

Concentration vs surfaces LSD 0.01 = 16.02

Figure (1): Micro-Vickers hardness testing machine (CV-400 DM).

Figure (2): Column chart illustrates the enamel hardness in (Kg .m-2) after MWCNTs application with DMSO in different concentration treatment.
Figure (3): Column chart illustrates the enamel hardness in (Kg .m\(^{-2}\)) after MWCNTs application with DMSO in different surfaces treatment

Figure (4): SEM: A: Unpolished sample without MWCNTs, B: Polished sample without MWCNTs, C: Unpolished sample with MWCNTs, D: Polished sample with MWCNTs in 5μm.

**Ethical Clearance**: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest**: The authors declare that they have no conflict of interest.

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20. Peter Atkin’s and Julio de. P. Atkin’s physical chemistry. 2006; 720-725.


Evaluation of CEA, CA19-9, and CA242 Tumor Markers in Patients with Colorectal Cancer by ELISA Technique

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Abstract

Colorectal cancer (CRC) is one of the common malignant tumors of gastrointestinal tract (GIT). A total of 40 patients with CRC were studied preoperatively and postoperatively while only 20 healthy persons were studied. Their ages ranged between 18-78 years. This study was carried out to investigate the possible association of some risk factors with CRC such as (age, gender, smoking, inflammatory bowel disease, and ulcerative colitis).

With application of ELISA technique, Serum levels of CEA, CA 19-9, and CA 242 was measured preoperatively and postoperatively. The results showed an increase in preoperative serum level of CEA in CRC patients when compared to both postoperative and control groups. There was a significant increase in the serum level of CEA (10.73 ± 4.01) comparison with the two other groups (postoperative 3.95 ± 1.55) and control (1.91 ± 0.27) at (P<0.05), but no significant differences between the two groups (postoperative and healthy) which have mean (3.95 ± 1.55 and 1.91 ± 0.27) respectively. Also showed a significant increase in serum level of CA19-9 in patients preoperative (23.28 ± 1.86), who were differed significantly from the mean of serum in patients postoperative (18.63 ± 1.40) and control group (19.11 ± 1.46) at (P<0.05), When compared the serum level of CA242 revealed a significant increase in serum level of patients preoperative (19.58 ± 3.46) as compared to postoperative (8.75 ± 1.77) and control group (9.85 ± 2.22) at (P<0.05).

Key words: Colorectal cancer, Biomarkers, Metastasis, CEA, CA19-9, CA242.

Introduction

Cancer is a disease that occurs when control is lost on cell division and growth as well as on the metastasis of abnormal cells. Reasons for cancer are both intrinsic, i.e. infections, smoking, radiant sources, and extrinsic, i.e. genetic and metabolic mutations, along with abnormal immune responses and hormone levels (1).

Cancers start in the cells that line the inside of colon (the longest fragment of the large intestine) and rectum (occupies the last several inches prior to the anus) (2). Stool blood, altered movement of bowel, continuous tiredness, nausea, vomit, malaise, anorexia, abdominal distension and losing of weight are among the main symptoms of CRC (3). Ageing and disturbed life style were reported to be the major reasons for developing CRC, with genetic abnormalities affecting only minor cases (4). Dietary influences which contribute to higher are red meat which is over processed, alcohol consumption, IBD (inflammatory bowel disease) (5). Tumor develops under strong influences from genetic and epigenetic disorders, while the survival can be best prognosis based on immunological microenvironment (6).

Materials and Method

Blood samples (5 ml) were collected from patients with colorectal cancer (preoperative- postoperative) healthy persons (control) by using plastic syringes. Serum samples were prepared by centrifuging the blood (5000 rpm for 15 min).

Tissue Biopsies: Biopsies were collected from tissues of CRC patients, followed by fixation in buffered formalin (10 %), embedding in paraffin wax, and finally staining by using hematoxylin-eosin. Tissue samples were collected from histopathological laboratories of Gastroenterology and Liver Diseases Teaching Hospital, Baghdad.
Immunological Assessments: Enzyme Immunoassay for Determination of Serum Tumor Markers by Using ELISA Kit: Quantitative determination of serum levels of three tumor markers (CEA, CA19-9 and CA 242) in both patient and control groups was performed by means of ELISA (Enzyme Linked Immunosorbent Assay).

Results

The study included 40 patients with colorectal cancer, 23 male (57.50%) and 17 female (42.50%), with an age range of 18-78 years. The selected patients were classified into two groups: the first group represented serum of preoperative patients and the second group was of serum of postoperative patients. These two groups were compared to the third healthy group which was used as control. The control subjects included 20 individuals, 11 males and 9 females with ages ranged between 24 and 58 years.

Distribution of patients with colorectal cancer according to different risk factors:
The age of the 40 adult CRC patients included in this study ranged between 18 and 78 years, the maximum numbers of colorectal cancer patients was found within age group more than 60 years (20: 50%), then 12 patients in the age group of 40-60 years (30%), and the minimum number was 8 patients in the age group of less than 40 years (20%). Results in Table (1) demonstrate that the total number of patients was 40, of which 23 were male (57.50%) and 17 were female patients (42.50%). This implies that colorectal cancer is more common in men than in women, with significant differences between the two genders at P<0.05, also the proportion of smoking included 21 smoker patients (52.50%) and 19 non-smoker patients (47.50%), with statistically non-significant difference.

Carcinoma groups grading of the present study revealed that well- differentiated adenocarcinoma (G1) was seen in 4 cases (10.00%) of the CRC group, while 30 cases (75.00%) of patients had moderate differentiated adenocarcinoma (G2), and 6 cases (15.00%) had poorly differentiated adenocarcinoma (G3 ).There were a significant differences at (P<0.05) among carcinoma groups according to their grading (Table 1).

In the current study, most of the patients had carcinoma as the type of colorectal cancer. Concerning the staging of the tumor, 6 patients (15.00%) had tumor that invaded the submucosa (T1), 12 (30.00%) had tumor that invaded the muscularis (T2), and 22 (55.00%) had tumor that invaded through the muscularis into the pericolorectal tissues (T3).

The results of our study showed that, among the 40 patients with CRC, 4 (10.00%) had genetic disease, 9 (23.00%) had polyp, 11 (27.00%) had ulcerative colitis, and 16 (40.00%) had diabetes mellitus type II. There were a significant differences at (P<0.05) among carcinoma groups according to the risk factors. Most of tumors are localized in different sites of colon. In this study, about 2 patients (5.00%) had tumor in the transverse colon, 3 (7.5%) in the right colon, 8 (20.00%) in the sigmoid colon, 12 (30.00%) in the rectum, and 15 (37.5%) in the rectosigmoid.

Serum Levels of CEA, CA19-9, and CA242 Tumor markers in patients with CRC According to their characteristics and risk factors:

Results in Table (32) exhibited a significant increase of serum levels CEA in patients with age range of 40-60 years as compared with those with age less than 40 years old. However, there were no significant differences between the serum levels of CEA in patients with age more than 60 years old and the other two groups at P<0.05.

Comparing serum levels of CA19-9 showed no significant effects of age in patients with age less than 40 years old, 40-60, and more than 60 years old.

Significant increases were recorded in serum levels of CA242 in patients with age range of 40-60 years as compared with serum levels in patients with age less than 40 years old and more than 60 years old at P<0.05. Also, the patients with age more than 60 had a significant increase in serum levels of CA242 in comparison with patients with age less than 40 years old at (P<0.05).

The results in table (2) show a significant increase in the serum level of CEA in females in comparison with that in males at P<0.05. Nevertheless, no significant differences were recorded in the serum levels of CA19-9 and CA242 between males and females. Also the results demonstrate a significant increase in serum level of CEA in smoker patients as compared with non- smoker patients at P<0.05. The statistical analysis showed non-significant difference in the serum levels of CA19-9 (and CA242 between smoker and non-smoker patients).
The results showed no significant difference in sera mean levels of CEA, CA19-9, and CA242 in patients with colorectal cancer in the T1, T2 and T3, while the relationship between serum levels of CEA and tumor grades of patients with colorectal cancer showed a significantly increased mean level of CEA in patients with G2 tumor as compared with G1 and G3 at P<0.05. There was no significant difference in mean levels of CEA between G1 and G3. The statistical analysis also showed non-significant difference in serum means levels of CA19-9 and CA242 in patients with G1, G2, and G3 (Table 2).

Table 1. Distribution of sample study of patients according to difference risk factors:

<table>
<thead>
<tr>
<th>Factors</th>
<th>Percentage (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group (year)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 40</td>
<td>8(20%)</td>
<td></td>
</tr>
<tr>
<td>40-60</td>
<td>12(30%)</td>
<td>0.0001 *</td>
</tr>
<tr>
<td>More than 60</td>
<td>20(50%)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>23 (57.50%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>17 (42.50%)</td>
<td>0.0372 *</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>21(52.50%)</td>
<td></td>
</tr>
<tr>
<td>Non-Smoker</td>
<td>19 (47.50%)</td>
<td>0.094 NS</td>
</tr>
<tr>
<td>Grade of Tumor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade I</td>
<td>4 (10.00%)</td>
<td></td>
</tr>
<tr>
<td>Grade II</td>
<td>30 (75.00%)</td>
<td>0.0001 *</td>
</tr>
<tr>
<td>Grade III</td>
<td>6 (15.00%)</td>
<td></td>
</tr>
<tr>
<td>Stage of Tumor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I</td>
<td>6(15.00%)</td>
<td></td>
</tr>
<tr>
<td>Stage II</td>
<td>12(30.00%)</td>
<td>0.0001 *</td>
</tr>
<tr>
<td>Stage III</td>
<td>22(55.00%)</td>
<td></td>
</tr>
<tr>
<td>Other diseases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genetic disease</td>
<td>4 (10.00%)</td>
<td></td>
</tr>
<tr>
<td>Polyp</td>
<td>9 (23.00%)</td>
<td></td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>11 (27.00%)</td>
<td>0.0001 *</td>
</tr>
<tr>
<td>Diabetes mellitus type II</td>
<td>16(40.00%)</td>
<td></td>
</tr>
<tr>
<td>Site of Tumor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transverse colon</td>
<td>2 (5.00%)</td>
<td></td>
</tr>
<tr>
<td>Right colon</td>
<td>3(7.5%)</td>
<td></td>
</tr>
<tr>
<td>Sigmoid colon</td>
<td>8 (20.00%)</td>
<td>0.0001 *</td>
</tr>
<tr>
<td>Rectum</td>
<td>12 (30.00%)</td>
<td></td>
</tr>
<tr>
<td>Rectosigmoid</td>
<td>15 (37.5%)</td>
<td></td>
</tr>
</tbody>
</table>

* (P<0.05), NS: Non-Significant.
Table 2: Serum Levels of CEA, CA19-9, and CA242 Tumor markers in patients with CRC According to their characteristics and risk factors:

<table>
<thead>
<tr>
<th>Studied groups</th>
<th>Mean ± SE</th>
<th>CEA in serum(ng/ml)</th>
<th>CA 19-9 in serum(U/ml)</th>
<th>CA 242 in serum(U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 40</td>
<td>2.88 ± 0.14 b</td>
<td>17.52 ± 2.04 a</td>
<td>4.16 ± 1.35 c</td>
<td></td>
</tr>
<tr>
<td>40-60</td>
<td>10.48 ± 5.67 a</td>
<td>25.09 ± 2.69 a</td>
<td>20.63 ± 4.64 a</td>
<td></td>
</tr>
<tr>
<td>More than 60</td>
<td>6.74 ± 2.43 ab</td>
<td>19.91 ± 1.37a</td>
<td>12.85 ± 2.34 b</td>
<td></td>
</tr>
<tr>
<td>LSD value</td>
<td>5.483 *</td>
<td>9.947 NS</td>
<td>6.808 *</td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>0.0330</td>
<td>0.171</td>
<td>0.0457</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2.62 ± 0.34 b</td>
<td>21.68 ± 1.46 a</td>
<td>15.07 ± 2.47 a</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>13.72 ± 4.92 a</td>
<td>19.98 ± 1.98 a</td>
<td>12.95 ± 3.43 a</td>
<td></td>
</tr>
<tr>
<td>LSD value</td>
<td>8.341 *</td>
<td>4.849 NS</td>
<td>8.194 NS</td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>0.0098</td>
<td>0.488</td>
<td>0.608</td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>11.36 ± 4.04 a</td>
<td>20.94 ± 1.87 a</td>
<td>15.14 ± 3.04 a</td>
<td></td>
</tr>
<tr>
<td>Non-smoker</td>
<td>2.89 ± 0.43 b</td>
<td>20.97 ± 1.42 a</td>
<td>13.09 ± 2.66 a</td>
<td></td>
</tr>
<tr>
<td>LSD value</td>
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<td>4.801 NS</td>
<td>8.112 NS</td>
<td></td>
</tr>
<tr>
<td>P-value</td>
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<td>0.986</td>
<td>0.615</td>
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</tr>
<tr>
<td>T1</td>
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<td>21.93 ± 2.72 a</td>
<td>16.70 ± 5.63 a</td>
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</tr>
<tr>
<td>T2</td>
<td>10.92 ± 5.72 a</td>
<td>22.57 ± 2.74 a</td>
<td>17.67 ± 4.58 a</td>
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</tr>
<tr>
<td>T3</td>
<td>6.69 ± 2.42 a</td>
<td>19.81 ± 1.38 a</td>
<td>11.57 ± 2.25 a</td>
<td></td>
</tr>
<tr>
<td>LSD value</td>
<td>8.993 NS</td>
<td>6.729 NS</td>
<td>11.370 NS</td>
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<tr>
<td>P-value</td>
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<td>0.610</td>
<td>0.478</td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>3.53 ± 1.09 b</td>
<td>23.22 ± 2.84 a</td>
<td>17.20 ± 4.45 a</td>
<td></td>
</tr>
<tr>
<td>G2</td>
<td>9.50 ± 3.17 a</td>
<td>20.17 ± 1.49 a</td>
<td>12.46 ± 2.48 a</td>
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</tr>
<tr>
<td>G3</td>
<td>3.03 ± 0.27 b</td>
<td>21.84 ± 2.69 a</td>
<td>18.30 ± 5.66 a</td>
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</tr>
<tr>
<td>LSD value</td>
<td>5.993 *</td>
<td>7.288 NS</td>
<td>12.314 NS</td>
<td></td>
</tr>
<tr>
<td>P-value</td>
<td>0.0420</td>
<td>0.566</td>
<td>0.368</td>
<td></td>
</tr>
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*(P<0.05), NS: Non-Significant
Means with different letters in same column differed significantly
Discussion

The development of colorectal cancer is caused by a combination of genetic and environmental factors. Epidemiology studies have revealed a number of risk factors for colorectal cancer including age, gender, smoking, family history of colon cancer or inflammatory bowel disease. Various individual characteristics or behaviors are included among the risk factors since they enhance the possibility to develop CRC. Age is one of the major risk factors for CRC. The risk of CRC increases with age. Several hypotheses have been suggested to find explanations for such a correlation between susceptibility to cancers and the progression in age, ageing of individuals is associated with increased and accumulated exposure to carcinogenic materials from the environment. In addition, the cellular mutation repair ability can be declined with increasing age (7).

Our study demonstrated that CRC is more common in men than in women. The incidence and mortality rates are higher by about 30 and 40%, respectively, in men as compared to women, while full explanation for this gender differences is not yet available, it is believed to be due to several factors such as higher exposure to carcinogens, differences in sex hormones, in addition to the interactions among the different factors (8).

Also the proportion of patients who smoked was higher than non-smokers; many studies have reported a higher risk of CRC among cigarette smokers, especially among those with a long history of smoking (9). Smoke from tobacco was reported to be one of the main carcinogen sources, such as heterocyclic amines, polycyclic hydrocarbons and nitrosamines. However, the evidence about the roles of these materials in development of colon cancer is insufficient (10).

Patients with adult onset type 2 diabetes show greater risk of CRC (11). Despite that the two diseases have several risk factors in common, such as obesity and a sedentary lifestyle, the correlation between them remains even after accounting for physical activity, body mass index, and waist circumference (12). Patients with chronic inflammatory bowel disease (IBD), who commonly suffer from inflammation in colon for extended periods, were reported to show a two-fold higher risk of CRC development (13). The extent, severity and duration of ulcerative colitis (UC), the most frequently reported form of IBD, were shown to associate with an elevated risk to develop cancer, UC is a chronic IBD with an unknown reason, mainly influencing the large intestine mucosa (14). Family risks of CRC are of a greater risk of developing the disease, with that history is mostly driven by genetic mutations and environmental factors (15). Genetics contributes to CRC risk by both gene-regulated pathways directly involved in disease development and inherited mutations accounting for about 10% of cases (16). The polyp is the classical model of colorectal cancer pathogenesis and it describes the phases of transition from benign tumors into colorectal cancer over many years, primary factors of polyp to CRC sequence are gene mutations, epigenetic alterations and local inflammatory changes (17).

Conclusions

1. In our study, we found that elderly age, male gender, smoking, and other infection, appeared to be the most possible association factors for colorectal cancer.

2. The appearance of tumor marker and their concentration are related to the genesis and growth of malignant tumors in patients, and it should correlate with tumor stages and grades.

3. There was a significant elevation in mean levels of CEA, CA19-9, and CA242 serum of CRC patients, when compared with healthy subjects.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they
have no conflict of interest.

**Funding:** Self-funding

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Knowledge about Emergency Contraception Pills among Primary Health Care Doctors in Baghdad\Al-Karkh Sector

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¹Family & Community Med Department/ College of Medicine/ Mustansiriyah University-Iraq,
²AlKakh health Center /Ministry of Health/Iraq

Abstract

Background: Unintended pregnancy is associated with an increased risk of problems especially when woman is not in her optimal health for childbearing. The problem of unintended pregnancy and its complication can be reduced by the use of emergency contraception.

Objective: To explore the knowledge of health care doctors in Baghdad/Al Karkh, Iraq regarding emergency contraception pills.

Participants and Method: A structured questionnaire was distributed to a total of 390 primary health care doctors (obstetrics and gynecology specialists, general practitioners and family physicians). The questionnaire contained two main domains: demographic characteristics and knowledge about EC.

Results: The majority of the sample were females 287 (73.6%), and the general practitioner (41.8%), family medicine specialists (34.6%). about one third of them were in age 40–49 years old, 72.1% of the physicians have no training about emergency contraceptive..

Conclusion: A deficit in knowledge shown by health care physicians lead to an insufficient use of emergency contraceptive pills methods.

Key words: Knowledge, Emergency Contraception, PHC physicians.

Introduction

Emergency contraception (EC) refers to methods of contraception that can be used to prevent pregnancy after sexual intercourse. These are recommended for use within 5 days but are more effective the sooner they are used after the act of intercourse, EC pills prevent pregnancy by preventing or delaying ovulation and they do not induce an abortion. EC cannot interrupt an established pregnancy or harm a developing embryo(1). Unintended pregnancy is associated with an increased risk of problems for the mom and baby. If a pregnancy is not planned before conception, a woman may not be in optimal health for childbearing(2). The problem of unintended pregnancy and its complication can be reduced by the use of emergency contraception (EC) (3). Unprotected intercourse that demand the use of emergency contraception include failure of barrier methods such as slippage, breakage or misuse of condom, sexual assaults, failed coitus interrupts, two or more consecutive missed oral contraceptive pills, or simply because intercourse was unexpected and therefore contraception had not been used(4).

Emergency Contraceptive pills can serve as a backup method and can reduce the number of unintended pregnancies and abortions. There are three types of ECPs: combined ECPs containing both estrogen and progestin, progestin-only ECPs, and ECPs containing an antiprogestin (either Mifepristone or Ulipristal Acetate). Progestin-only ECPs have now largely replaced the older combined ECPs because they are more effective and cause fewer side effects. Although this therapy is commonly known as the morning-after pill, the term is misleading; ECPs may be initiated sooner than the morning after—immediately after unprotected intercourse—or later—for at least 120 hours after unprotected intercourse. Combined ECPs contain the hormones estrogen and progestin. The hormones that have been studied extensively in clinical trials of ECPs and found to be highly effective and well
The antiprogestin mifepristone approved for use in many countries for early first trimester medication abortion and it is highly effective for use as emergency contraception, with few side effects (delayed menstruation following the administration of mifepristone is one notable side effect) (8).

The aim of the present study is to assess the knowledge about EC pills among healthcare physicians at Baghdad Al Karkh/Iraq

**Participants and Method**

A cross sectional survey was conducted among health care physicians in Baghdad Al Karkh District during the period from Nov 2017 to Mar 2018. The studied population included 390 physicians (Obstetricians & Gynecologists, General Practitioner, Family physicians) in primary health care (PHC) centers. They were recruited by convenient selection method. The questionnaires were delivered to participant personally by the researcher himself at their work place to ensure completing the questionnaire instantaneously. Anonymity of participants was insured.

**Questionnaire:** Structured questionnaire were developed as per objectives of the study, this questionnaire was adopted from study made in 2005 for Exploring Knowledge, Attitudes and Practices of EC in PHC provider Tribhuvan University with modification (9). Each participant was asked to complete the questionnaire which consist of two elements; Sociodemographic information “gender, age, specialty, marital status, years of experiences and formal training” and EC knowledge in PHC “17 main questions and 6 sub questions”.

**Statistical Analysis**

The data were coded and each questionnaire assigned with a serial identifying number then entered by the researcher into the computer using Statistical Package for Social Sciences (SPSS) version 24. Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range (minimum-maximum values). The significance of difference of different percentages (qualitative data) were tested using Pearson Chi-square test with application of Yate’s correction or Fisher Exact test whenever applicable. Statistical significance was considered whenever the P value was equal or less than 0.05.

<table>
<thead>
<tr>
<th>Gender</th>
<th>No</th>
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</thead>
<tbody>
<tr>
<td>Male</td>
<td>103</td>
<td>26.4</td>
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<tr>
<td>Female</td>
<td>287</td>
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<table>
<thead>
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<th>Specialty</th>
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<td>General practitioner</td>
<td>163</td>
<td>41.8</td>
</tr>
<tr>
<td>Family Medicine</td>
<td>135</td>
<td>34.6</td>
</tr>
<tr>
<td>Obstetric &amp; Gynecologists</td>
<td>36</td>
<td>9.2</td>
</tr>
<tr>
<td>Others (Pediatrics, ENT, Rotator…etc.)</td>
<td>56</td>
<td>14.4</td>
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</table>

<table>
<thead>
<tr>
<th>Age (years)</th>
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<td>20---29</td>
<td>36</td>
<td>9.2</td>
</tr>
<tr>
<td>30---39</td>
<td>107</td>
<td>27.4</td>
</tr>
<tr>
<td>40---49</td>
<td>132</td>
<td>33.8</td>
</tr>
<tr>
<td>50---59</td>
<td>95</td>
<td>24.4</td>
</tr>
<tr>
<td>=&gt;60</td>
<td>20</td>
<td>5.2</td>
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<table>
<thead>
<tr>
<th>Marital status</th>
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</thead>
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<td>Unmarried</td>
<td>70</td>
<td>17.9</td>
</tr>
<tr>
<td>Married</td>
<td>320</td>
<td>82.1</td>
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</table>

<table>
<thead>
<tr>
<th>Years of working experience (years)</th>
<th>No</th>
<th>%</th>
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<tr>
<td>0-9</td>
<td>108</td>
<td>27.7</td>
</tr>
<tr>
<td>10-19</td>
<td>114</td>
<td>29.2</td>
</tr>
<tr>
<td>=&gt;20</td>
<td>168</td>
<td>43.1</td>
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</table>
Table 2: The distribution of PHC physician according to their knowledge about EC (n=390)

<table>
<thead>
<tr>
<th>Know the name of EC (n=320)</th>
<th>No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Have you ever hear about EC</td>
<td>320</td>
<td>82.1</td>
</tr>
<tr>
<td>The source of information</td>
<td></td>
<td></td>
</tr>
<tr>
<td>College study</td>
<td>212</td>
<td>66.2</td>
</tr>
<tr>
<td>Mass media</td>
<td>13</td>
<td>4.1</td>
</tr>
<tr>
<td>Continuous medical education in PHCC</td>
<td>8</td>
<td>2.5</td>
</tr>
<tr>
<td>MOH</td>
<td>77</td>
<td>24.1</td>
</tr>
<tr>
<td>Others</td>
<td>10</td>
<td>3.1</td>
</tr>
<tr>
<td>Know the name of EC (n=320)</td>
<td>244</td>
<td>76.4</td>
</tr>
<tr>
<td>Method is: Same as combined oral contraception pills (COCs) and/or high dose of hormones (Ulipristal acetate (UPA))</td>
<td>283</td>
<td>88.4</td>
</tr>
<tr>
<td>Intra uterine device (Cupper T)</td>
<td>160</td>
<td>53.7</td>
</tr>
<tr>
<td>Have EC in the PHC center</td>
<td>84</td>
<td>26.2</td>
</tr>
</tbody>
</table>

Table 3: The distribution of PHC physician according to knowledge about mechanism of action, effectiveness, indication, safety, and testing for pregnancy before EC (n=320)

<table>
<thead>
<tr>
<th>Know mechanism of action</th>
<th>No</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>The mechanism of Action</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prevent implantation</td>
<td>55</td>
<td>25.3</td>
</tr>
<tr>
<td>Prevent implantation &amp; ovulation</td>
<td>68</td>
<td>31.3</td>
</tr>
<tr>
<td>Induces abortion</td>
<td>81</td>
<td>37.4</td>
</tr>
<tr>
<td>Do not know</td>
<td>13</td>
<td>6.0</td>
</tr>
<tr>
<td>Effectiveness of EC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preventing pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very good (&gt;95%)</td>
<td>78</td>
<td>24.4</td>
</tr>
<tr>
<td>Good (75-90%)</td>
<td>198</td>
<td>61.8</td>
</tr>
<tr>
<td>Fair (50-74%)</td>
<td>17</td>
<td>5.4</td>
</tr>
<tr>
<td>Don’t know</td>
<td>27</td>
<td>8.5</td>
</tr>
<tr>
<td>Safety profile of EC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Very safe</td>
<td>22</td>
<td>6.9</td>
</tr>
<tr>
<td>Safe</td>
<td>249</td>
<td>77.9</td>
</tr>
<tr>
<td>Cause health problems</td>
<td>35</td>
<td>10.8</td>
</tr>
<tr>
<td>Not sure</td>
<td>14</td>
<td>4.4</td>
</tr>
<tr>
<td>Know the indication of EC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>When contraception method has been used</td>
<td>144</td>
<td>57.3</td>
</tr>
<tr>
<td>Condom rupture</td>
<td>227</td>
<td>90.2</td>
</tr>
<tr>
<td>Condom used perfectly</td>
<td>143</td>
<td>57.0</td>
</tr>
<tr>
<td>Condom slippage</td>
<td>206</td>
<td>81.8</td>
</tr>
<tr>
<td>Correct coitus interruption</td>
<td>121</td>
<td>48.2</td>
</tr>
</tbody>
</table>
Results

Table 1 presents the demographic characteristics of the health care providers who completed the study questionnaire; the majority of them were females 287 (73.6%) while the rest were males (26.4%). Among them were general practitioner (41.8%), family medicine specialists (34.6%), obstetric & gynecological specialists (9.2%) and from other specialties (Pediatric, ENT, rotator … etc.) (14.4%). Regarding their age, about one third of them were in age 40–49 years old (33.8%), 27.4% were 30-39 years, 24.4% were 50-59 years, and 9.2% were 20–29 years while about 5.2% were 60 years and above. Most of the PHC physician were married (82.1%) with working experience less than 10 years in 27.7% of them, 10–19 years (29.2%) and 20 years and above representing about half of them (43.1%).

The term EC was known by 82.1% of physician with their source of knowledge from college study (66.2%), MOH (24.1%), mass media (4.1%), continuous medical education in PHCC (2.5%) and others (brochures, reading, journals, internet e-mails….etc.) in 3.1% (Table 2).

Among 320 PHC physician who heard about EC 244 (76.4%) knew the trade name of the available types of EC. The majority of those 320 PHC physician mention that EC were same as COC pills and/or high dose of hormones “Ulipristal acetate” (UPA) (88.4%)

Table 3 reveals that 67.9% of PHC physician know the mechanism of action of EC as inducing abortion (37.4%), preventing implantation and ovulation (31.3%), only preventing implantation (25.3%), or no exact mechanism is known (6.0%).

Among 320 PHC physician, 41.3% of them think that the client no need to do pregnancy test before take EC and the rest either they trust their clients and consider need for pregnancy test before EC. More than half of the PHC physician (61.8%) consider the effectiveness of EC in preventing pregnancy is good, 24.4% as very good, while the rest either did not know (8.5%) or assume it as fair (5.4%). Regarding safety, more than two-thirds of physician (77.9%) believed that EC have a safe profile (Table 3). Table 3 also demonstrates that larger number of PHC physician have known the right indication of EC (78.7%) as follow, condom rupture 90.2%, condom slippage 81.8%, for woman had been a victim of sexual assault 81.1%, IUCD expulsions 66.8%, miscalculation of the periodic absent method 61.2%, while those physician who mention the wrong indication was as follow, when contraception method has been used 57.3%, condom used perfectly 57.0%, correct coitus interruption 48.2%.

Discussion

Family planning programs in Iraq are unfortunately limited and based only on little consultancy clinics within PHCCs and the private clinics . The use of contraceptive methods by Iraqi women reached a rate of 56.1% . Present study showed that 82.1% of PHC physicians heard about emergency contraception (EC). This finding is lower than results of Batur et al study in Turkey of 53.7% of PHC workers who heard about levonorgestrel EC. However, rate of hearing about EC by our study is higher than that reported by Mandiracioglu et al study in Turkey of 53.7% of PHC workers who heard about EC. Differences in hearing about EC proportions between studies might be attributed to discrepancy in quality of medical education and training of physicians between different countries. Current study revealed that 76.4% of PHC physicians know the name of EC. This EC knowledge rate is close to knowledge rate of family physician working in PHC reported by
Abdulghani et al(14) study in Pakistan of 71% and that of health care workers reported by Zeteroğlu et al(15) Study in Turkey of 74%.

However, knowledge rate of EC in present study is higher than results of Harrison cross sectional study in Nigeria which stated that only 45% of medical doctors could correctly defined the emergency contraception(16). On other hand, knowledge of current study PHC physicians regarding EC is lower than that of 87% knowledge rate of physicians reported by Lo et al(17) study in Hong Kong. These differences in knowledge regarding EC between studies may be due to different reasons like differences in governmental interest in family planning programs, information given as part of the medical curriculum lectures in medical colleges or during postgraduate training and continuing medical education. Most of PHC physicians (88.4%) had knowledge in EC method of combined oral contraception pills (COCs) and/or high dose of hormones (Ulipristal acetate (UPA)). This finding is higher than results of Oriji and Omietimi study in Nigeria(18) which reported that although, 98% of medical doctors had good knowledge in EC, 58% of them could not recognize the EC types. More than half (63.8%) of PHC physicians believed that combined pills is the best choice. Current study showed that 67.9% of PHC physicians knew the emergency contraception mechanism of action and 37.4% of them thought that mechanism by induced abortion. This proportion of knowledge is higher than results of Ebuehi O et al study in Nigeria which found that 48.8% of health care providers have good knowledge in EC mechanism of action. Knowing mechanism of EC action is essential for physicians practice, effectiveness and dealing with complications(19).

This study showed that 24.4% of PHC physicians believed that EC is very good and 61.8% believed that it is good. These findings are close to Results of Lawrence et al(20) study in USA which revealed that 89% of physicians believed that EC and access to EC would lower the rate of intended pregnancy. Knowing emergency contraception indications was observed among 78.7% of PHC physicians in present study. The highest knowledge regarding the indications was for condom rupture (90.2%) and lowest for correct coitus interruption (48.2%). These findings are close to results of Fok study in USA which reported that 74.7% of physicians had proper knowledge regarding the indications of EC(21).

In conclusion, a clear deficiency in PHC physicians’ knowledge about EC pills methods which leads to an insufficient use of EC pills methods.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** Self-funding

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Relationship of Vitamin D with Some Electrolytes in the Serum of People with Rheumatoid Arthritis in the City of Samarra

Adeeb Mahfooth Farag Al-Samaria¹, Kader Abdullah Shanak Al-Esawi², Esraa Ali Abdul Kareem Al-Samaria³, Dhamer Ismael Madab AL-Khazraji⁴

¹Iraq/Ministry of Education / Directorate General of Salah Uddin Education, ²Iraq/Ministry of Scientific Research-University of Fallujah, ³Iraq/Ministry of Scientific research-University of Samaria, ⁴Iraq/Ministry of Education / Directorate General of Salah Uddin Education

Abstract

The study was conducted on 50 samples of 30 patients with rheumatoid arthritis. The samples were divided into 15 females, 15 males and 20 control group (healthy) without any disease. The sample was divided into 8 females and 12 males. 80 years) and collected samples from Samarra General Hospital and outpatient clinics of the city. The blood was then collected from the healthy and sick people and separated by centrifugation. Biochemical variables were measured (vitamin D, glutathione, Mallon dialdehyde, uric acid).

The results of the current study showed a significant decrease in the level of vitamin D in the serum of people with rheumatoid arthritis compared to healthy people, and the results showed a significant decrease in the level of glutathione in the serum of people with rheumatoid arthritis compared to healthy people, with no significant differences in Level of Mallon dialdehyde. We also note a significant increase in the level of uric acid in the serum of people with rheumatoid arthritis compared to healthy people.

Key Words: - rheumatoid arthritis, Vit. D, Oxidative stress

Introduction

Arthritis is often used to indicate any disorder affecting the joints¹, including symptoms such as Jointstiffness, Joint pain, Redness, Swelling, difficulty in movement, and may affect other organs. Rheumatoid arthritis, rheumatoid arthritis, gout (²) osteoporosis usually occurs with age and affects the joints near the ends of the fingers at the base of the thumb and neck and below. Back, knees and hips. (³) Rheumatoid arthritis is an autoimmune disorder that often affects the shoulders, hands, knees and feet and also affects cartilage, tendons, and bones through swollen vertebral tissue (²).

Rheumatoid arthritis is a multifaceted form of disease whose causes, symptoms, and treatment are different (⁴). Rheumatoid arthritis is a common health problem affecting millions of people worldwide, leading to higher health care costs (⁵) Of the world’s population, with a prevalence rate of 1-0.5% (⁶), while in Colombia it was 0.9% (⁷).

The treatment of arthritis is focused on alleviating the symptoms and improving the ability of the joints to function. It is sometimes necessary to experiment with various treatments or to combine different treatments with each other in order to be able to determine the best treatment for the patient. There are many drugs used to treat inflammation (⁸) Including analgesic drugs, nonsteroidal anti-inflammatory drugs or some dietary supplements such as Glucoseamine and Chondroitin sulfate. Studies have shown that corticosteroids use longer and higher, leading to peripheral joints. (⁹) There are many environmental factors that increase the risk of rheumatoid arthritis and the most serious risk is smoking. Previous studies have shown that smoking increases the development of the disease, especially in patients who have a positive result of ACP (¹⁰). Environmental factors are not just smoking, but factors such as food, alcohol, vitamin deficiencies, viruses and bacteria. (¹¹) These factors are all outside the body and have no genetic basis, so they are called non-genetic factors (¹²). Epstein Barr Virus (EBV) was also associated with rheumatoid arthritis. There was an abnormal increase in the number of B lymphocytes infected with this virus in the blood of the rheumatoid arthritis patient. This virus stimulates the
production of antibodies including the rheumatic factor (13).

Vitamin D is a type of fat-soluble vitamin, and sun exposure is the main source for the body’s needs of this vitamin, so it is called vitamin sun rays, which is different from the rest of the vitamins not necessary from food sources for this reason enough exposure to radiation sun for 10 to 15 minutes a day on sunny days, and two to three times a week to get vitamin D requirements in the majority of people (14).

Vitamin D is of biological importance to the body of the organism. It is essential for balance of calcium, bone growth and regulation of the immune system. Its deficiency can cause rickets disease, osteoporosis, osteoporosis and muscle weakness. Vitamin D deficiency is linked to cancer, cardiovascular disease and schizophrenia. Arthritis, type 1 diabetes, IDDM, psoriasis and vitiligo (15), as well as a decrease in risk associated with increased mortality (16) and increased risk of breast cancer (17).

Oxidative stress is defined as the imbalance between free radicals (ROS), Reactive Nitrogen Species (RNS), and antioxidants, which are important indicators of many pathological conditions, including atherosclerosis (18), as well as disorders Heart, blood vessels and arthritis (19) and an imbalance between it and antioxidants may also cause an increase in blood pressure Hypertension (20).

Since vitamin D may be related to rheumatoid arthritis, the current research target for measuring vitamin D in patients with rheumatoid arthritis.

Material and Method

Collection of specimens

The study was conducted on 50 samples of 30 patients with rheumatoid arthritis. The samples were divided into (15 females, 15 males) and 20 control groups (healthy) without any disease. The sample was divided into 8 females and 12 males. 80 years) and collected samples from Samarra General Hospital and outpatient clinics.

Blood collection

Collect about 6 cm³ of the blood of healthy and sick people and is divided according to the type of test. The blood is placed in the Jell tubes. It has an airtight cover, free of anticoagulant, leaving the blood at 25 °C until it coagulates and then placed in the centrifuge for 10 minutes at 3000 cycles/minute and then the serum was obtained and then placed in small test tubes and kept in the refrigerator at a temperature of 20 - M for the purpose of measuring the biochemical variables, including (vitamin D, glutathione, Mallon dialdehyde, uric acid).

Estimation of Vitamin D

The level of vitamin in the serum was assessed using the Kit kit and manufactured by German company Human by method (21).

Estimation of antioxidant

Glutathione-GSH was estimated by Sedlak, Tietz (22,23). The concentration of uric acid in the serum was estimated using the enzymatic method using several prepared analyzes prepared by the Tunisian Biomegrheb (24).

Estimation of Oxidative stress

Determination of the level of Mallon dialdehyde-MDA in Guidet (25).

Statistical analysis

The results of all tests were analyzed using statistical program SPSS14, mean Mean and standard deviation (SD), and the special differences between the infected groups and the control group were determined using T.Test (26).

Result and Dissection

Measuring the level of vitamin D

The results showed a significant decrease in serum vitamin D levels in people with rheumatoid arthritis compared to healthy subjects as shown in Figure 1.
Vitamin D deficiency may lead to rheumatoid arthritis. Lee (2016) (27) points out that vitamin D decreases in people with rheumatoid arthritis, which is consistent with current research results. Azzen (2012) (28) found that vitamin D has a role in reducing the risk of rheumatoid arthritis, as there are many immunosuppressive effects including vitamin D as there is a potential relationship between vitamin D deficiency and autoimmune disease (29).

Vitamin D has been studied as an important and potential measure of the causes of many diseases, including rheumatoid arthritis. (30) The reason for vitamin D deficiency may be due to insufficient absorption of the vitamin D, which may be associated with exposure to sunlight (31). Athanassion (2012) (32) also noted that vitamin D deficiency is very widespread in patients with rheumatoid arthritis and that its deficiency greatly increases the disease. There should also be vitamin D supplementation to prevent osteoporosis as well as relieve pain for patients with rheumatoid arthritis. (33) (2017), Hamad, noted that vitamin D levels were lower in rheumatoid arthritis patients as there should be comprehensive research studies on the role of vitamin D in the development of rheumatoid arthritis and its relationship to disease activity. Vitamin D has the function or function of regulating immunity associated with the potential effectiveness of vitamin D receptors. These include the treatment of many diseases, including rheumatoid arthritis and psoriasis, as well as many diseases (34).

**Glutathione concentration measurement**

Result shows the mean ± morbidity of the GSH level as the results showed a significant decrease in the level of GSH in the serum of people with rheumatoid arthritis in healthy people as in Figure (2).

In its study, Al-Maamory (35) reported a significant decrease in the level of glutathione in the serum of people with rheumatoid arthritis compared to healthy subjects, which is consistent with the results under study.

The low level of glutathione is due to several reasons, including the increase in the rate of consumption, which is one of the most important non-enzymatic antioxidants in the removal of free radicals and their products, transforming from the effective form to the ineffective form. The sulfur group in the GSH structure is a low-efficient factor. Between sulfur and hydrogen (SH) and the power of kin between carbon and hydrogen (CH) in the free radicals so they protect the cellular membranes from damage to free radicals.

One of the reasons for the low level of GSH is a deficiency in the raw materials of its structure, in particular the adjuvant enzyme (the reduced form) nicotine amide adenine deoxyribonide phosphatase, which is the catalyst for the action of the enzyme GRd, which works to restore the effective form of the collation of the ineffective form (36).

**Measurement of Mallon dialdehyde**

Result shows the mean ± standard deviation of the level of Mallon dialdehyde. The results showed that there was no significant difference in the concentration of Mallon dialdehyde in serum group of infected and healthy people as in Figure (3).

The results are not consistent with both Abbas (2011) (37) and 2008 (AL-Maamory)(35), who noted in their study that there was a significant increase in the level of MDA in the serum of people with rheumatoid arthritis.
Measurement of uric acid

Result shows the average ± standard deviation of uric acid level. The results showed a significant increase in serum uric acid concentration in people with rheumatoid arthritis compared to healthy subjects as shown in Fig. 4.

Sheania (2011) reported a significant increase in the level of uric acid in the serum of rheumatoid arthritis patients and this is consistent with the results under study. Das (2014) (39) indicated a rise in serum uric acid levels in people with rheumatoid arthritis.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Study of Thyroid Hormones for Vitiligo Patients in AL-Anbar Governorate

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Abstract

Vitiligo is a common acquired depigmentation disorder characterized by the loss of functional skin and mucosal melanocytes the reported incidence is 0.5% to 2% worldwide. Its pathogenesis is mostly consider autoimmune and this pigmentary disorder is strongly associated with autoimmune thyroid disorders. To evaluate the serum thyroid hormone in vitiligo patients of Al-Anbar governorate and to compare the results with other external studies. The study group included 80 patients with vitiligo, and 40 healthy volunteers. Blood thyroid hormone was determined using Monobind kits from reliable USA company. Their ages ranged form 1 to 70 years. Family history of vitiligo was positive in a percentage of (30%) of the patients. The mean levels of serum thyroid hormone (T3, T4) in patients with vitiligo were found to be no significant difference than those of healthy individuals. The TSH level in patient with vitiligo were found to be significantly higher than those of healthy individuals. This study strengthens the relationship between the thyroid hormones intake, formation, and metabolism with the pathogenesis of vitiligo. Therefore it is concluded that vitiligo patients should be evaluated for thyroid hormones.

Key Words: Vitiligo, Thyroid hormone, Autoimmune thyroid, T3, T4, TSH.

Introduction

Vitiligo is an acquired hypopigmentary skin disorder affecting the population of worlds without discrimination of race, age, gender and ethnic background(1). It is characterized by the formation of white patches and these patches associated with the loss of local melanocytes. Vitiligo involves the progressive loss of epidermal melanocytes and sometimes hair follicle melanocyte(2). Vitiligo is more significant in population of dark skinned individuals, due to its pigmentary disfigurement and has major impact on quality of life of patients(3). It produces social stigma in the affected individuals and is often confused with leprosy or other socially terrifying infectious diseases. It is a non-contagious disorder and the most commonly acqurid hypomelanosis(4). The lesions in vitiligo patients are most commonly found on the body areas that are exposed to sun like hands, arms, faces, feet etc. Patients suffering from vitiligo may have premature graying of the scalp hair and eyebrows along with the appearance of white patches on the skin. Vitiligo is linked with simultaneous occurrence of other autoimmune diseases as well as psychosocial difficulties (5)(6)(7). In certain cultures, patients having vitiligo are regarded as social outcasts(3). Several reports have suggested associations between vitiligo and a variety of other autoimmune diseases, including thyroid conditions, alopecia areata, type 1 diabetes mellitus, pernicious anemia, and rheumatoid arthritis. Autoimmune thyroid diseases are common in patients with vitiligo(8). Studies have reported that the incidence of thyroid disease is 0-52% in patients with vitiligo, and that 3% to 90% of vitiligo patients have antithyroid antibodies(9)(10). Therefore, routine screening for thyroid dysfunction is recommended for patients with vitiligo(11).

Patients and Method

A total 80 patient with vitiligo were enrolled. Half number of healthy individuals with matching ages were included as controls. The samples were collected from the patients during their visiting to dermatological clinic of Dr. Abdullah salih Alhasan in Al-Anbar governorate. The ages of the patients ranging between 1-70 years old from both sexes. Many questions were asked to the patients about his name, age, accommodation, occupation, chronic diseases, family history, time of
infection, the presence of psychological disturbances, smoking, most common diet, most common drinks, spiritual questions (prayer), time of disease exacerbation, and the factors that exacerbate vitiligo to avoid the interferences with the other diseases, and to find a cause for this disease. All of patients and healthy individuals were not smokers, have no any chronic diseases, and not alcoholic drinkers. A total of (10 ml) of venous blood was drawn in sterile syringe and centrifuged to separate the serum and then stored at -45°C until begin used. The estimation of triiodothyronine (T3), thyroxine (T4), and thyroid-stimulating hormone (TSH) levels were done by using a Microplate Enzyme Immunoassay(12)(13)(14) from Monobind company (made in USA).

Results

The study included a total of 120 persons. Among them 80 had vitiligo (30 male and 50 female) and 40 were healthy controls (8 male and 32 female). Their ages ranged from 1 to 70 years. Family history of vitiligo was positive in a percentage of (30%) of the patients. The duration of disease ranged between 1 month to 20 years. History of seasonal variation of disease was positive in (60%) patients. Out of these (10%) noticed exacerbation of disease in winter while (50%) in summer season. Bad emotional state exacerbates of about (70%) of vitiligo patients, while the other (30%) dose not affected. This study showed that there is no relationship between the occupation, accommodation, most common diet, most common drinks, and spiritual side and vitiligo. In the patient’s group T3 and T4 were not significant statistically than those in control group. while TSH was significantly higher than those in control group. The results are depicted in Table 1. The values are reported as mean ± SD and 95% confidence interval. For statistical analysis between groups paired t test was used. Pearson test was used for correlation analysis. The levels of each marker were compared between the study groups and control group, using SPSS computer package. P values of ≤ 0.05 were considered significant. The table above shows that T3 and T4 are not significant difference between patients and controls (P ≥ 0.05). While TSH is significantly higher in patients in a comparison with controls (P ≤ 0.05).

Table 1: the mean value ,S.D, t-value, and p-value of the parameters were tested.

<table>
<thead>
<tr>
<th>No.</th>
<th>parameters</th>
<th>factor</th>
<th>Mean ± SD</th>
<th>t-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T3 ng/ml</td>
<td>patient</td>
<td>1.03±0.30</td>
<td>-0.429</td>
<td>0.669</td>
</tr>
<tr>
<td></td>
<td></td>
<td>control</td>
<td>1.05±0.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>T4 µg/dl</td>
<td>patient</td>
<td>7.69±1.86</td>
<td>0.000</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>control</td>
<td>7.69±1.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>TSH µIU/ml</td>
<td>patient</td>
<td>3.33±2.27</td>
<td>2.920</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>control</td>
<td>2.23±0.96</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion: Thyroid functional disorders and autoimmune thyroid diseases have been reported in association with vitiligo, and it seems that the incidence of clinical and subclinical thyroid involvement the incidence of clinical and subclinical thyroid involvement is more common in vitiligo patients than controls(15)(16)(17). Many researches about vitiligo and thyroid disease have been done with different results, one of these Mubki et al.,(2017)(18) showed that thyroid functional abnormalities were generally found more in vitiligo patients were approximately 1.6 times more likely to have abnormal TSH than control. The mean TSH level was overall higher in the vitiligo group. Both high TSH and low TSH levels were seen more frequently in vitiligo patients. The vitiligo group had significantly higher prevalence (5%) of primary hypothyroidism (high TSH and low T4) as compared to the control group. Alissa et al.,(2011)(19) and Akay et al.,(2010)(20) showed the predominance of females among vitiligo patients can be attributed to the fact that females are more conscious
about their cosmetic appearance and thus more likely to seek medical attention. Vitiligo seems to be commonly associated with autoimmune diseases. Two studies have reported associated autoimmune disease in (19%) and (23%) of vitiligo patients.(21)(22).

One of the most commonly reported associations is thyroid disease, especially Hashimoto’s thyroiditis.(23). The reported prevalence of thyroid disease in the literature ranged from (4%) to (21%) to even higher in other studies.(22)(24)(25).

Conclusions

From our results we conclude that a strong relationship is found between the thyroid hormones and the pathogenesis of vitiligo.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

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Detection of Quorum Sensing Signal Molecules and Identification of espB and Crt4 genes among Biofilm Forming of Citrobacter freundii

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¹Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Iraq

Abstract

150 samples from different clinical sources were collected from October 2018 to March 2019 from three hospitals in Maysan: Al Sadr General Education Hospital, Al Zahrawi Surgical Hospital and Maternity and Child Hospital to demonstrate the spread and distribution of Citrobacter freundii in several hospitals in Maysan. All isolates were identified based on morphological characteristics and biochemical tests. Results were confirmed by Api 20 E and Vitek 2 compact. A total of 14 isolates (9.3%) of 150 were found to be Citrobacter freundii. The isolates were considered as acute diarrhea in children, UTI, burns, wounds and most frequent ear swabs. PCR results showed that the LuxR 428bp genes were present in the Citrobacter freundii bacteria identified by previous diagnostic methods and this confirms the accuracy of the tests and methods used to determine this type.

Keywords: Citrobacter freundii; espB and Crt4 genes; Quorum sensing signal

Introduction

Citrobacter, a genus of the Enterobacteriaceae family, Gram-negative, facultative anaerobic bacteria that look as coccobacilli or rods (1). Citrobacter spp. are motile using their peritrichous flagella, can ferment mannitol with making of H2S, and can use citrate as their single source of carbon (2),(3). Citrobacter spp. are uncommon opportunistic nosocomial bacteria can cause urinary tract, hematologic, or neonatal infections (e.g. meningitis, sepsis, general bacteremia); intra-abdominal sepsis; brain abscesses; or pneumonia (4),(5). Citrobacter spp. infections can be mortal with 33-48% overall death rates being reported including 30% for children(6),(7). Children and immune deficiency, elderly, or weakened patients are at risk of infection (2), (9). Citrobacter spp. is prevailing worldwide, as it is a part of the normal intestinal flora of humans (10),(11). Less well known species that have also been implicated in foodborne disease like some strains of Citrobacter spp. (notably C. freundii), Klebsiella spp., Providencia spp. Enterobacter spp. and Proteus spp., may occasionally cause what is often described as opportunistic gastroenteritis (12), this study aimed to isolation and identification of C. freundii from chicken meat samples using cultural and molecular techniques.

Method and materials

Samples collection

150 samples from different clinical sources were collected from October 2018 to March 2019 from three hospitals in Maysan.

Isolation

each sample was inoculated on the Salmonella shigella (SS) agar medium, the plates were left to solidify at room temperature, and then were incubated at 37 °C for 24-48 hours. Later the grown colonies were further investigated

Identification

The Citrobacter isolates were identified to the level of species using the traditional morphological and biochemical tests (13). The identification of isolates was confirmed by vitek2 compact system.

Cultural characteristics on selective and differential media.

SS, MacConkey and Xylose lysine deoxycholate (XLD) agar
The organisms were cultured on S.S agar media and incubated overnight at 37°C. The colonies of C. freundii appear with black center after 24hrs incubation period. The suspected colonies of C. freundii cultured on MacConky media, the positive result appears pink (Lactose fermenters) after 24hrs incubation period, pale colonies further incubated for 24hrs to identify the (late lactose fermenters). The selected colonies were cultured on Xylose lysine deoxycholate agar, after 24hrs, the positive result appeared as yellow colonies (13).

Eosin Methylene Blue (EMB) agar

In order to differentiate Citrobacter from E.coli, the lactose fermenter isolates were subcultured on EMB for 24hr. at 37°C. Brown colonies were the positive result (14).

Identification of bacteria by Vitek 2 compact system.

Vitek 2 compact was used to identify the bacterial isolates. It is a compact system of two parts, Instrument and computer. The reagent cards have 64 wells that can each contain an individual test substrate. Substrates measure various metabolic activities such as acidification, alkalinisation, enzyme hydrolysis, and growth in the presence of inhibitory substances.

Identification of Bacteria by PCR

DNA Extraction

Genomic DNA was isolated from Bacteria according to the protocol of Wizard Genomic DNA Purification Kit, Intron. A PCR reaction with a specific primer (Table-1).

Table 1-Primers sequences

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Sequences</th>
<th>Tm°C</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LuxR-F</td>
<td>GCACGGATTACATCATTA</td>
<td>49.3</td>
<td>428</td>
</tr>
<tr>
<td>LuxR-R</td>
<td>GCACGGATTACATCATTA</td>
<td>49.3</td>
<td></td>
</tr>
</tbody>
</table>

For LuxR gene was performed to identify C. freundii (Table-2).

Table 2-Reaction mixture

<table>
<thead>
<tr>
<th>PCR master mix</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA template</td>
<td>5 µL</td>
</tr>
<tr>
<td>Green master mix</td>
<td>12.5 µL</td>
</tr>
<tr>
<td>Forward Primer10pmol</td>
<td>2.5 µL</td>
</tr>
<tr>
<td>Reverse Primer10pmol</td>
<td>2.5 µL</td>
</tr>
<tr>
<td>Free nuclease water</td>
<td>2.5 µL</td>
</tr>
<tr>
<td>Total</td>
<td>25 µL</td>
</tr>
</tbody>
</table>

(25µl) of PCR amplification mixture contained (12.5 µl) Master mix, (1 µl) forward primer, (1 µl) reverse primer, (8.5 µl) nuclease free water, and (2 µl) DNA template. The protocol for PCR condition was initial denaturation 95°C for 5 min. denaturation 95°C for 30 sec., annealing 60 °C for 40 sec., extension 72 °C for 1 min. and final extension 72 °C for 7min.

Results and Discussion

Bacterial Isolation and Identification

Twenty five chicken meat samples were collected from local markets in Baghdad city. Citrobacter was detected in 3 samples, were all samples cultured on S.S. agar for initial isolation, after incubation at 37°C for 24 hr ; different types of bacterial isolates appeared on S.S. agar, of them: small pale flattened colonies with
black center due to their ability to produce H2S on S.S agar, then these colonies sub-cultured on MacConkey, XLD and EMB to differentiate Citrobacter from Salmonella because both of them are H2S. Citrobacter is lactose fermenter on MacConkey agar appeared as pink colonies while Salmonella is pale colonies (Non lactose fermenter) on XLD Citerobacter appeared as yellow colonies while Salmonella appeared as red colonies with black center. After incubation period; lactose fermenter (pink) on MacConkey and yellow colonies on XLD while on EMB they were brown in colour, these were depended as Citrobacter. To confirm the primary identification Gram stain was performed to examine the microscopic properties which were Gram negative bacilli. The ability of Citrobacter to produce urease enzyme was detected using urease test in order to differentiate it from the genus Proteus which was urease producer while Citrobacter isolates were non urease producers. Thus depending on colonial morphology; bacterial isolates were identified as Citrobacter Figure-1 (A, B, C, D) and (Table-3) showed these biochemical tests used to identify Citrobacter as described by(15),(16).

Figure 1-Different selective and differential media cultured with Citrobacter spp. after incubation at 37°C for 24 hr.

A. Pale colonies with black center on S.S. agar
B. Small pink (Lactose fermenter) colonies on MacConkey agar
C. Yellow colonies on XLD agar
D. Brown colonies on EMB.
Table 3-Result of biochemical tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growing on MacConkey agar</td>
<td>Dry Pink colonies</td>
</tr>
<tr>
<td>Growing on EMB</td>
<td>Not forms green metallic sheen</td>
</tr>
<tr>
<td>Gram stain reaction</td>
<td>Gram negative bacteria</td>
</tr>
<tr>
<td>Urease</td>
<td>Non urease producer</td>
</tr>
<tr>
<td>S.S agar</td>
<td>Pale colonies with black center</td>
</tr>
<tr>
<td>XLD agar</td>
<td>Yellow colonies</td>
</tr>
</tbody>
</table>

To confirm the identification of Citrobacter spp. Vitek 2 compact system was depended and the result showed that the isolated bacteria in this study was Citrobacter and the species freundii

In order to confirm the identification of Citrobacter to species level LuxR gene amplification was performed using monoplex PCR technique, 1.5 % agarose gel electrophoresis was used to detect the positive result as shown in Figure-2.

(Figure 2) Amplified PCR products of LuxR gene (428 bp): Agarose gel electrophoresis, ethedium bromide stained, 1.5 % agarose, electrophoresed in 75 volt for 2 hrs and photographed under ultraviolet trans-illuminator. M: The DNA molecular weight marker (100 bp ladder) and 1: the amplified PCR product of LuxR of C10 isolate of Citrobacter freundii.

One of the most gorgeous likely uses of 16Sr RNA gene sequence informatics is to offer genus and species or tax identification for isolates (17). Although 16SrRNA gene sequencing is highly valuable in regards to bacterial classification (18). PCR products were exposed to direct sequencing, both strands of PCR products were sequenced with an automatic sequencer. Sequences were analyzed with the Basic Local Alignment Search Tool (BLAST) in National Center for Biotechnology Information (NCBI).

Conclusion

The following conclusions were obtained from this study:

- The prevalence and distribution of Citrobacter spp. in some Maysan hospitals shows a relatively low percentage in its distribution.
- The dominance species of Citrobacter was Citrobacter freundii.
- All isolates of Citrobacter freundii produced Bioflim formation by Congo red agar, Christensen method and micro-titer plate assay.
- The effect of different temperature and pH values on Citrobacter freundii growth showed that best growth temperature was 37°C and the best growth pH for growth was 7.
• These data are of great significance as the signal molecules aid in biofilm formation which in turn confer various properties of pathogenicity to the clinical isolates including drug resistance. The use of quorum sensing signal blockers to attenuate bacterial pathogenicity is therefore highly attractive, particularly with respect to the emergence of multi antibiotic resistant bacteria.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** Self-funding

**References**


The effect of Beta-amino Butyric Acid in Levels of Interleukin4 & Interleukin 10, Complement Proteins C3 & C4 and Immunoglobin IgM in Males Rats Sprague Dawley Infected with Pseudomonas aeruginosa

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Abstract

B–amino butyric acid (BABA) non protein amino acid has effect on some immunological parameters of male rats Sprague Dawley species infected with pseudomonas aeruginosa. this study include 25 animal divided into five groups A, B, C, D and E each group contain five animals, A, B and C groups injected with three concentration of amino acid solution (25 mg/ml, 50 mg/ml and 75 mg/ml) with dose (0.2 g/kg, 0.4g/kg and 0.6 g/kg) respectively, intra peritoneal weekly for six weeks, group D (first control group) and E (second control group) injected with normal saline. At the fifth week the four groups A, B, C and D exposed to pseudomonas aeruginosa, while group E did not exposed to infections after the termination of period of treatment, The blood samples were collected to do the immunological tests, the statistical analysis P<0.05 results showed BABA did not enhance sensitivity through measuring interleukin IL4, and significant increase of IL10 and complement protein C4, while BABA didn’t have a negative effect on the complement protein C3 activation and increase the level of immunoglobulin IgM.

Keywords: Beta-aminobutyric acid, Sprague Dawley rats, Pseudomonas aeruginosa, Interleukin IL10, IL4, Complement proteins C3 & C4, Immunoglobin IgM.

Introduction

The bacteria considered important causes of disease, that include the most of diseases that infect humans in the word and the most important prefix and present and futurism epidemiological diseases(1), Pseudomonas aeruginosa considered important bacterial species due to its content of virulence factor that increase its pathogenicity(2). It’s considered dangerous for patients especially patients with wounds & burn inflammation, that it can invade blood supply and cause Septicemia especially patients with Immunodeficiency(3). P. aeruginosa characterized by its resistance to antibiotics and disinfectant, and this cause big problem in whole the word and the main cause of this resistance is random use of antibiotics without restriction(4), due to continues antibiotic residue lead to development of bacterial resistance to antibiotics(5), this what make researchers to new applications by using chemical materials other than antibiotics(6), that enhance body resistance to bacterial infections, the nominated materials to replace antibiotics is organic acids(5), Which are include many acids like Non-protein amino acids that present in the nature, more than 1000 non protein amino acid produced by plants and microorganism and other sources(7), these components did not have specific functions in the nature, but they noticed have many physiological functions In vivo studies(8), For the example, Gamma-amino butyric acid (GABA) work as stimulator and regulator of immune response that it boosted the Innate immunity and Adaptive immunity, that work on improvement of body resistance against bacteria by activation of Phagocytosis, and matureness of Macrophages and improve its response against microorganism(9), and activation of T cells and B cells, increase antibodies like IgG and IgM(10), GABA increase of Anti-inflammatory cytokines like interleukin IL10 and suppression of Pro-inflammatory cytokines and work on stimulation of Apoptosis of cells that damaged by pathogenic factors(11), also the Non-protein amino acids β-amino butyric acid (BABA) was improved recently that make hematological and immunological changes, that increase...
red blood cells, white blood cells, hemoglobin, packed cells volume and lymphocyte, also cause increase in immunoglobulin IgG\(^{(12)}\).

Due to rarity of studies about the effect of this acid in animal aspect with its availability in plant aspect, and due to BABA known with its ability to stimulate plant resistance against wide range of causative agent like viruses, bacteria, fungi and worms\(^{(13)}\), also have significant effect in plant resistance to insects\(^{(14)}\).

So this study aimed to identification of ability Non-protein amino acids BABA to induction of rats resistance to \(P. \text{aeruginosa}\).

**Materials and Method**

**Preparation of amino acid solution**

Amino acid solution prepared by dissolving 0.5g, 1g and 1.5g from acid in 20ml of normal saline to obtain the first concentration C1 (25mg/ml) and second concentration C2 (50mg/ml) and third concentration C3 (75mg/ml) respectively with continues mixing until acid dissolving then the wanted dose prepared according to animal body weight, with percentage of 0.2g/kg from first concentration C1 and 0.4g/kg from second concentration and 0.6 g/kg from third concentration.

**Preparation of the lab animals**

Experimental animals consist of 25 of Sprague Dawley male rats with age 10 to 12 weeks and weight ranged between (225-300) g, divided into five groups each groups five animals divided as following:

1- Group A first concentration group C1(25mg/ml)
2- Group B second concentration group C2 (50mg/ml)
3- Group C third concentration group C3 (75mg/ml)

The groups above injected with amino acid intrapersonal\(^{(15)}\) weekly for six weeks.

4- Group D first control groups: injected with normal saline intrapersonal.

The four groups above exposed to infection with \(P. \text{aeruginosa}\) Bactria.

5- Group E second control group this group injected with normal saline intrapersonal and did not exposed to infection with \(P. \text{aeruginosa}\).

**Animal infection with \(P. \text{aeruginosa}\)**

The animal infected after the fifth weeks of amino acid BABA injection at age (15-17) weeks with weight ranged between (265-380) g. the isolation activated with by obtain part of bacterial cultivation to inoculate nutrient broth tube and incubate with 37 C for 24 hours. After appear of the bacterial growth decimal dilution done by using normal saline and 1 ml of each diluent was obtained and inoculates on nutrient agar to count the colonies in the suspension. Rats anesthetized with Chloroform. Than shaved the area down the head from the back\(^{(16)}\). Wounded the skin deeply without damage the subcutaneous muscles, after sterilization by using forceps and Scissor, skin biopsy obtained in about 6mm diameter of five animal groups (A, B, C,D and E), by using Micropipette 200µm of suspension with turbidity (2 x 10\(^6\)) putted on the wound to contaminate it\(^{(17)}\) in four animal groups that include group A, group B, group C and first control group D, while second control group E did not contaminated with bacteria.

The animals examined for its nutrition and activity and wound healing after expend of seven days of infections the physiological and immunological tests were done.

**Serological tests**

Immunological tests were carried out on the serum of the rats to detect the level of IL-4, IL-10, C3, C4 and IgM in all groups. Blood was obtained from posterior vena cava\(^{(15)}\).

3 ml of blood was withdrawn in plastic tubes free of anticoagulant to obtain the serum using centrifuge 3500 rpm for ten minutes. The serum samples were kept at -20°C until the time of testing using (Elisa kit/ Elabscience Biotechnology Inc. /USA) and (Genus Kit/ Genrui Biotech Inc./China)

**Statistical Analysis**

The statistical analysis done by using One-way ANOVA test with statistical analysis program SPSS 22 edition and the mean calculated and stander deviation, finding LSD value from Multiple comparisons table at level of significance 0.05.
Results and Discussion

Effect of BABA on level of interleukin IL4

The results of statistical analysis as in table 1 which show the significant differences in LSD values between groups, and figure 1, show that A, B, C and D (three concentration group and first control group) that exposed to bacterial infection was recorded significant increase in level of interleukin IL4 compare with second control group that did not exposed to bacterial infection.

![Figure 1 show the means of interleukin IL4 values](image)

From the above the level of IL4 that increased in infected groups as the results of infection with bacteria not resulted from BABA amino acid that appeared the mast cells are activated to interact with wound repairing and wound healing at the same time with infection by microorganism, that have vigilant factors that disturb wound healing mechanism as Exotoxins of P. aeruginosa this cells stimulated presented in the subcutaneous tissue that migrate to the wound site and secrete its content due to expose to bacteria products, the studies conclude that the mast cells activated by its exposer to bacterial toxin or lipopolysaccharide (LPS) bacterial wall content, also fined that the mast cells that present in peritoneal layer execrate Histamine as the results of P. aeruginosa bacteria in rats, and it’s an important source of early response cytokines like IL4 that necessary to begin the immune response and inflammation of the host ageist invaders\(^{(18)}\), and considered main resource of IL4\(^{(19)}\), that the mast cells activated by bacteria that cause diseases also in the case of absence of antibodies and information and in this case be the source to generate IL4 and other regulating cytokines to do Non-opsonization reactions\(^{(18)}\), the mast cells response to many stimulators independently without interaction of IgE and release its component, in this situation did not considered hypersensitivity response because IgE not unclouded in the interaction, and this explain support absence of signs of hypersensitivity in animals.

Effect of BABA on level of IL10

The results of statistical analysis P<0.05 as in table 1 and figure 2 show the A group record significant increase in level of IL10 in compare with E and D control groups, also group C record showed significant increase in level of IL10 in compare with E second control group.

![Figure 2 show the means interleuken 10 value](image)

By notest the figure 2 showed that the mean of IL10 value elevated in animal groups treated with BABA amino acid in compare with E and D control groups but just group A recorded significant increase in compare with control groups E and D, and group C recorded significant increase in compare with E second control group, while group B did not recorded any significant increase for unclear causes, and this may explane the level of IL10 in group A and the dose 0.2 g/kg of BABA amino acid and this dose needed for production of IL10 with significant level and this agree with what mentioned by\(^{(11)}\), that the GABA which analogous with BABA, increase the production of IL10 significantly in case of colon inflammation induced in mice, that GABA work as stimulator for antiinflammatory cytokines and inhibit Pro-inflammatory cytokines and enhance Apoptosis for cells that damaged with diseases factors.

Effect of BABA levels of complement proteins C4 and C3 and immune globulin IgM

Results of Statistical Analysis P<0.05 showed as appeared in table 1 and figure 3 that the five group A, B, C, D and E did not show any significant increase in level of complement protein C3, with non-significant sharp decrease of C3 mean values in group A, also decrease the value of C3 in second control group E, and the decreased values indicate the activation of complement protein\(^{(20)}\).
Figure 3 show the means of complement protein C3 values

While the complement protein C4 the results of statistical analysis P<0.05 were showed as in table 1 and figure 4 that the group A recorded significant increase in level of C4 in compare with group B, while other groups did not recorded any significant differences, and this increase may cause by bacterial infections or may be due to the 0.2 g/kg dose of BABA acid is the optimal dose to increase complement proteins C4 significantly, as appear in figure 4 that showed means of complement proteins C4 values.

Figure 4 show the means of complement protein C4 values

From the compares between figure 3 and figure 4 noticed there are negative correlation between C3 and C4 values and this difference may return to activation of complement C3 to activate Alternative pathway or Lectin pathway as an innate immune response to infection, and lead to activation B cells to production of antibodies to activation of Classical pathway\(^{(21)}\). And this indicates that the BABA amino acid did not have negative effect to prevent complement C3 activation process. And have positive effect in activation of classical pathway of complement system that depends on C4 protein.

While measurement of immunoglobulin IgM, the results of statistical analysis (P<0.05) as in table 1 and figure 5, that the group A was recorded significant increase in level of IgM in compare with group B, C and E, that the 0.2 g/kg lowest dose suitable to production of IgM and this results did not agree with\(^{(21)}\) mentioned that the increase dose increase production of immunoglobulin IgG and this may returned to interactions with bacterial infections or may results category of antibody and molecular compound of each one.

Figure 5 show the means of immunoglobulin IgM values

By compare of figure 4 and figure 5 noticed that presence of clear positive proportions between complement protein values C4 and immunoglobulin IgM, and this may lead to activation classic pathway of complement system, the interaction of B cells that produce immunoglobulin IgM that stimulate activate of classic pathway of complement system, and activation

Table 1 show the LSD values

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>IL4</th>
<th>IL10</th>
<th>C3</th>
<th>C4</th>
<th>IgM</th>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Group B</td>
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<td>.166</td>
<td>.113</td>
<td>.048*</td>
<td>.003*</td>
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<tr>
<td>Group C</td>
<td>.471</td>
<td>.646</td>
<td>.300</td>
<td>.158</td>
<td>.006</td>
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<tr>
<td>Group D</td>
<td>.073</td>
<td>.038*</td>
<td>.383</td>
<td>.326</td>
<td>.105</td>
</tr>
<tr>
<td>Group E</td>
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<td>.007*</td>
<td>.951</td>
<td>.079</td>
<td>.008*</td>
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<tr>
<td><strong>Group B</strong></td>
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<td></td>
</tr>
<tr>
<td>Group A</td>
<td>.146</td>
<td>.166</td>
<td>.113</td>
<td>.048*</td>
<td>.003*</td>
</tr>
<tr>
<td>Group C</td>
<td>.445</td>
<td>.344</td>
<td>.559</td>
<td>.529</td>
<td>.737</td>
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<tr>
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Table 1 shows the LSD values

<table>
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<tr>
<th>Group</th>
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<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
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<tbody>
<tr>
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<td>.445</td>
<td>.260</td>
<td>.004*</td>
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<tr>
<td>Group B</td>
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<td>Group D</td>
<td>.158</td>
<td>.729</td>
<td>.652</td>
<td>.701</td>
<td>.326</td>
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<tr>
<td>Group E</td>
<td>.06*</td>
<td>.737</td>
<td>.187</td>
<td>.989</td>
<td>.105</td>
</tr>
</tbody>
</table>

* means difference is significant in LSD values between groups at the 0.05 level

**Conclusions**

Current study assures that the non-protein amino acid BABA have clear positive effect in accelerate of wound healing process and decrease inflammation, the serological tests P<0.05 ensure that the BABA did not generate hypersensitivity in animal of experiment. BABA have positive effect in production of IL10 then it has role in regulation of immune response, BABA did not stop activation of complement system but lead to stimulation of B cells to production of IgM antibody and interaction between Innate immunity and Adaptive immunity.

**Acknowledgement:** I would like to thank Dr Othman Makki Sagheer and Dr Atheer Khalaf for their scientific support for this research. I would also thank Dr Yasser Mufeed and Ms. Anwar Yousef for their efforts to complete this research.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** Self-funding

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The Toxic Effect of Cadmium Chloride on Lung Function and Tissue and the Protective Role of Pomegranate Seed Oil in Female Rabbits

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Abstract

The experiment was designed to determine the protective role of pomegranate seed oil on lung function and tissue against cadmium chloride toxicity.

In this experiment, 20 animals (rabbit females) were used. Lung efficiency was measured by measuring the size of red blood cells and hemoglobin, as well as studying the histological effects of cadmium chloride against lung tissue and the protective role of pomegranate seed oil. The results showed that the treatment of animals with cadmium chloride at a dose of 5 mg / kg bw for 30 days led to a significant increase in packed cell volume (PCV) and hemoglobin (Hb) compared to control group.

The treatment of animals with cadmium chloride has also shown that there is no possibility of distinguishing the alveolar of lung (AV) with the presence of focal infiltration of the inflammatory cells with a marked thickening of the wall of the blood vessel (TW) with blood congestion (CON). Pomegranate seed oil improves most of the negative effects caused by cadmium chloride.

Key word: Cadmium chloride, Pomegranate seed oil, Packed cell volume (PCV), Hemoglobin (Hb).

Introduction

Cadmium (Cd) and lead (Pb) are ubiquitous and non-biodegradable pollutants representing a great concern to human health. Both metals are naturally distributed, but industrial development has dramatically increased their concentrations in the environment (1). The World Health Organization (WHO) has published a list of 10 chemicals or groups of chemicals of concern for human health, which includes Cd (2). The toxicity of cadmium has attracted the attention of researchers in different countries of the world due to its toxic effect on the cells and tissues of the body. The focus of the researchers has been on reducing the effect of effective oxygen species and free radicals causing their formation, as well as increasing interest in reducing the impact of industrial pollutants in the environment in which we live (3). So researchers have sought to use a large number of plant-derived pharmaceutical products in traditional medicine or to use their extracts because of their useful properties. The plants are rich in a wide range of secondary compounds such as flavonoids, tannins, alkaloids and others (4). Mentioned that the damaged central nervous system and DNA or cancer progression appeared as consequences of Cd exposure. Cadmium also causes severe soft tissues and bone damages (5). After absorption, Cd and Pb are distributed in the organisms via red blood cells or proteins. A major amount of Cd in red blood cells is bound to high-molecular-weight proteins, while a minor amount is bound to hemoglobin (6). The hematopoietic system is one of the most sensitive systems and blood represents not only the mode of transportation, but also the critical toxicity target of Cd and Pb (7). Cadmium causes lung damage, pulmonary fibrosis, emphysema, and inflammation in human and experimental animals. Cadmium may also adversely affect the lungs by decreasing the viability or modifying the function of individual lung cells (8). The toxic mechanisms responsible for cadmium-induced lung cell damage are not well understood. One study (9).

The use of extracts from medicinal plants and their effective non-food chemical compounds has a preventive and therapeutic effect for many disease cases and has little or no side effect compared to chemically manufactured laboratory drugs (10). Pomegranate, Punica granatum L.,
is an ancient medicinal food plant which natively grows from the Himalayas in northern India to Middle East but has also been cultivated and naturalized in many other regions including Mediterranean, Southeast Asia, tropical Africa, and American Southwest (11). In addition to extensive uses of pomegranate in folk medicine of many cultures, pharmacological studies have shown that pomegranate fruit preparations have antioxidant and anti-inflammatory, (12) antimicrobial, (13) anticancer, and chemopreventive (14). Pomegranate seeds are rich in sugar, unsaturated- polyunsaturated fatty acids, vitamins, polysaccharides, polyphenols and minerals (15). In particular, pomegranate seed oil contains high levels of phenolic compounds which is punic acid, punicalagins (PNG), as well as important fatty acids such as linoleic acid, gallic acid and elagic acid (16). Ellagic acid is a polyphenol compound with antioxidant and anti-proliferative properties that also exists in many other fruits and plants such as raspberries, pecan nuts and strawberries. These components demonstrate anti-inflammatory and antioxidant effects by inhibiting the expression of pro-inflammatory enzymes and cytokines (17); anticarcinogen, antioxidant (18) anti-inflammatory, antimicrobial (19, 20), which are free radical scavenging compounds (21). Pomegranate is also rich in vitamins and minerals (22).

**Material and Method**

2.1. Experimental Design: After acclimatization, animals were randomly divided into four groups: Group 1 – Animals were given distilled water and kept as control.

Group 2 – Animals were given Pomegranate seeds oil (0.8 ml)/ kg b.w. for 30 days (23).

Group 3 – Female rabbits were treated at a dose of cadmium chloride 6 mg/kg b.w for 30 days, which promised an infected control. (24).

Group 4 – Animals were given Pomegranate seeds oil (0.8 ml)/ kg b.w. with cadmium chloride 5 mg/kg b.w for 30 days.

2.2. Hematological Examination:

Blood samples were taken from the retroorbital venous plexus of rats. The two blood samples were collected one with EDTA for hematological analysis and other for separate serum for biochemical analysis. Erythrocyte count (RBCs) was performed using improved Neubauer Hemocytometer and Gower's fluid as a diluting fluid according to (25). PCV% was determined by using microhematocrite centrifuge and microhematocrite capillary tubes method according to (26).

2.3. Histological study

Lung tissue samples were fixed in 10% formalin since 24 hours, dehydration by ethyl alcohol in increasing concentrations (70%, 80%, 95%, 100% and 100%), clearing with xylene and then embedded with paraffin. When analyzed, all paraffinembedded tissue was sectioned at 5 µm and stained with Hematoxilin and eosin. These specimens were examined under a light microscope at 40X magnification power. Corresponding digital images were captured for later analysis (27).

**Results**

![Figure (1) The lung segment control group shows pulmonary bronchioles (BR) and the alveoli (AV) within the lung tissue in its natural form H & E 400X](image-url)
Figure (2) The lung segment The treatment of cadmium chloride shows that it is not possible to distinguish alveoli (AV) with focal infiltration of inflammatory cells (IF) H & E 100X

Figure (3) The lung section The cadmium chloride treatment shows the renal variability (AV) with a marked thickening of the wall of the blood vessel (TW) with blood congestion (CON) H & E 100X

Figure (4) Lung section group treated with pomegranate seed extract showing normal the alveoli (AV) H & E 400X
Discussion

It is noted from the results above that treatment of animals with cadmium chloride at a dose of 6 mg / kg body weight for 30 days led to significant increase (P≤0.05), in blood volume and hemoglobin compared to control group, in consonance with found . ( 28) . While did not agree with (29), Who used a single dose with cadmium chloride (0.1mg / kg) . It was found that the treatment of mice With cadmium for 21 days led to a significant decrease in the value of Hb . while the protective role of pomegranate seed oil against chloride Showed a significant decrease in blood volume and hemoglobin compared with the cadmium chloride-treated animals group . The hematopoietic system is one of the most sensitive systems and blood represents not only the mode of transportation, but also the critical toxicity target of Cd and Pb. (7). Both metals may lead to anemia by various mechanisms (30). Cadmium and Pb are transported to the liver, in which they can cause damage and disturbed function. Liver damage can be confirmed by histopathological findings and is often accompanied by increased blood enzyme levels and reduced protein synthesis (31).

Changes in the size of red blood cells The cause is believed to be a physiological condition to compensate for the lack of oxygen in the body because of the thickening of the gas exchange membrane between the alveolar of lung and the blood and these changes lead to increase the formation of red blood cells from the reservoir of body (32) . This problem is also very important and interesting because there are many reports in literature that cadmium can result hypoxia (33). That’s why it is topical to research metabolical effect of cadmium ions and hypoxia and find out the biochemical and morphological changes of
blood indices of rats under cadmium loading as blood is a substance of organism that reacts on irritation from environment very quickly.

The decrease in RBCs count during the chronic treatment might be resulted from severe anemic state or haemolysing power of heavy metals (cadmium chloride) particularly on the red cell membrane. this agreed with (34). The reduction in erythrocytes count might be due to the destruction of mature erythrocytes and the inhibition of erythrocytes production . It is also noticed from the tissue sections of the lung tissue that the treatment of animals with cadmium chloride led to the possibility of distinguishing the pneumonia (AV) with the presence of central infiltration of inflammatory cells with a clear thickening in the wall of the vessel (TW) with congestive blood (CON), in consonance with(35,36).

Lung tissue is one of the main targets of cadmium toxicity (37), and the respiratory system is affected severely by the inhalation of cadmium-contaminated air. Shortness of breath, lung edema, and destruction of mucous membranes as part of cadmium-induced pneumonitis have been described Cadmium causes lung damage, pulmonary fibrosis, emphysema, and inflammation in human and experimental animals. Cadmium may also adversely affect the lungs by decreasing the viability or modifying the function of individual lung cells (8).

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**Conflict of Interest:** The authors declare that they have no conflict of interest.

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A Biometric Assessment of a Combined Topical Levofloxacin, Retinol, Cloxacillin and Ascorbic acid Against Facial Acne

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Abstract

Acne vulgaris is a common pilosebaceous lesion affects skin over the face and upper chest. It has about 14 million incidence rate that cost 100$ for each case. In this study, biometric outcomes of the combined retinol, levofloxacin, cloxacillin, ascorbic acid and the hydrocarbon base vehicle on different types of acne lesions in human were investigated. Twenty one person were included in two groups; test (N =10) and control (N = 11) with follow up of objective software based biometric analysis parameters including: keratolysis induction, redness reduction and healing of infected lesion. The test formula revealed a significant keratolysis induction as compared with control. Eight out of 10 individuals with the test formula had keratolysis in comparison with 2 out of 11 had no keratolysis in controlled group, P-Value = 0.005. Similar effects were obtained in redness reduction (redness reduction ratio induced with test formula = 2.5 with confidence interval CI over 0.95) and impetiginization healing at P < 0.05. From the overall results, the combined retinol, levofloxacin, cloxacillin, ascorbic acid and the hydrocarbon base vehicle showed significant improvement in biometric outcomes of facial acne lesions.

Key words: Facial acne vulgaris, RGB image processing, keratolysis, impetiginization

Introduction

Acne vulgaris is a common dermatological lesion characterized by progressive popular to nodular skin lesion over the face and sometimes upper chest, back and shoulders (1,2). Studies estimated 14 million presentations to the clinic suffering from acne vulgaris with 85% of cases were between 15-17 years of age(3-5). It is presented in different forms: close, open, and black and white. It affects both sexes with average age incidence(6). Acne vulgaris lesions cost 100$ for each case in average(7).

In Iraq and nearby countries prevalence of acne was 13.1% among skin diseases(8,9). Acne vulgaris has multifactorial causation. Androgen hyperactivity especially dihydrotestosterone(10,11) expression of binding factor (binding protein II and proline rich protein I) to Propionibacterium acne, excess inflammatory response to P. acne (12) and microcomidos formation with closure of sebaceous duct and accumulation of sebum (13,14). Different cytokines and chemotaxis factors are noticed in excessive amount in acne lesion like IL1, IL12, IL8 and prostaglandins (15). Of the most common complications that are associated with this dermatological disease, facial and neck impetiginization and scar.

Factors that predispose to acne complications include hormonal hypersensitivity, hormonal imbalance, bacterial infections, age, weight, cosmetics and skin histopathological typing (16,17). The most commonly used antiacne treatments include keratolytics, antibacterial and peeling agents (18-20). However, a fraction of acne lesions are refractory to treatment that mandate more therapeutic researches.

The objective of this study is that to assess biometric outcomes of the combined topical formula of;
levofloxacin, disintegrating agent, retinol, hydrocarbon mineral carboxylate, cloxacillin, pH buffering agent, ascorbic acid and vehicle base on different types of acne lesions in human.

**Samples, Materials and Method**

**Study design**

Controlled clinical trials of acne vulgaris was designed to exclude:

- Age: 25yr < age < 13yr
- Medications and antiacne intake
- Chronic medical illnesses like HT, DM and hormonal disturbances
- Weight: 70 kg < wt < 50 kg

Prerequisites of medical ethics (according to Geneva and Helsinki declarations) submitted to local ethical committee.

- All of the used drugs and excipients are FDA approved for safety and efficacy
- All of the used drugs were passing phase I (i.e. not used as a first time)
- Benefit is prevailed
- All individuals were informed about study design and expected side effects
- All individuals permission, autonomy and consents have been obtained
- All of the used drugs were through topical route of administration
- All individuals were prior tested for any skin hypersensitivity and side effects.
- All individuals names, private secrets, faces were respected and insured
- All rights were reserved.
- All were free to be subjected to medical treatment or test treatment.

**Material**

1) *Hydrocarbon cream o/w*

Of multiple esters; Myrsticate, glycol monostearate, Na palmitate, Na stearate, isopropyl alcohol and water 5.05 ml

2) **Active ingredient and vehicle base:**

- Retinol (Egypt, product date and expiry date; PD-ED: 2013-2015)
  Package of 200000 IU. The used dose in designing the topical test formula against acne was 0.05%.

- Cloxacillin (Ajanta, India, PD-ED: 2014-2015). The used dose was 0.1%

- Levofloxacin (Pharma International, Jordan; PD-ED: 2014-2015). The used dose was 0.1%.

- Ascorbic acid (Merck, PD-ED: 2013-2015). The used dose was 0.1%

- Hydrocarbon base was used as a vehicle of Myrsticate, glycol monostearate, Na palmitate, Na stearate, isopropyl alcohol and water 5.0 mL.

*Constituents of the combined antiacne formula;*

(Levofloxacin, disintegrating agent, retinol, hydrocarbon mineral acyls, cloxacillin, pH buffering agent, ascorbic acid, vehicle base)

*Constituents of the blank controlled formula;*

(Hydrocarbon base+ mineral oils+ pH buffer, disintegrating agent)

Pharmaceutical analysis is done to assess formula pH and physical consistency was assessed with PHELECT computerized pH meter electrode (USA). pH was buffered around 6.3.

**Methods**

Acne sample selection

The lesion to be monitored with biometric method was not randomly selected

Criteria of selection

- More prominent lesion in the face is selected to be monitored.
- Type of lesion was randomized (to include all presenting lesion types for further analysis and concluding the overall effect on all types)
Monitoring was done in conserved temperature but different ambient lighting because this was processed and normalized by software.

Selection of blank treated and test treated was randomized.

There was no person with any application of either test or blank to insure ethical requisites. On the other hand, no person was with standard medical treatment.

**Methods of observation and analysis**

Places of application were therapeutic research lab/Kufa College of medicine for assessment of students. Private lab for assessment of the volunteers out of the college as well.

**Ambient conditioning**

Lighting was objectively evaluated to be controlled

Ambient temperature has averaged 22-25 ºC

A prior imaging of the lesion was taken then after 4 day of the treatment another image was acquired for MIP.

MIP was a mathwork 2013a image processing blockset design by Dr Hussein AbdulKadhim for processing and analyzing RGB and lesion pattern for:

1- RGB shift
2- Lesion dimension
3- Pattern of lesion response (keratolysis, impetiginization, dimentions and bevel).

**Treatment Mode**

The entire face was messaged with the test combination and left overnight then morning washing. Another application was done for 1hr duration to be washed prior to attending time.

**Biometric Analysis Method**

The biometric set composed of a Microsoft combined thermographic tissue camera with MIP for objective image analysis.

Analyzing histogram vector RGB and lesion in form
of red pixelate shift was objectively monitored and data were analyzed statistically with spline interpolation and risk reduction at 0.95 C.I. Discrete data was analyzed with chi square test at P < 0.05. Statistical software packages were Matlab 2013 statistical toolbox and Minitab 2014 statistics.

**Results**

1- Findings and analysis of keratolytic activity of the test combined topical formula.

**Chi-Square Test: test; control**

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<thead>
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<th>control</th>
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<tr>
<td><strong>Total</strong></td>
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<td>11</td>
<td>21</td>
</tr>
</tbody>
</table>

Chi-Sq = 8.025; DF = 1; P-Value = 0.005

1 cells with expected counts less than 5.

Figure 2: Number of individuals who showed keratolysis in response to the applied test formula as compared to those used a blank cream base after 14 days of treatment. So that 8 out of 10 individuals with the test formula had keratolysis in comparison with 2 out of 11 had no keratolysis in controlled group. P-Value = 0.005.

2- RGB analysis findings for acne lesions

Color model interpolation and the mean red value estimation with redness reduction ratio.

In control group:

MIP red value was reduced from 240-200 = 40

In test group:

MIP red value was reduced from 250-150 = 100

So the redness reduction ratio of test formula = 2.5 at C.I. 0.95.

**Figure 3**: Mean values of red bins by which the MIP had shifted after 14 days of treatment. This was a direct indicator of acne reactivity in response to treatment.

3- Lesion impetiginization response

**Chi-Square Test: test; control**

<table>
<thead>
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Chi-Sq = 4.531; DF = 1; P-Value = 0.033

2 cells with expected counts less than 5.
Discussion

A trial of assessment of topical antiacne formula has a significant consideration since it can be reasonably safe alternate to systemic administration of drugs for prolonged period.

The reason behind selecting combined active ingredients in designing the test formula was to induce synergism since P. acne, S. aureus and S. pyogen are rapidly emerging resistance against the commonly used antimicrobials\(^{21,22}\) and to potentiate peeling with retinol and keratolysis with ascorbic acid\(^{23,24}\). This principle causes augmentation of antiacne effect.

However this study concerned a limited number of population and needs for further confirmation in larger samples. Overall clinical and biometric outcomes are best to be included in further studies.

The clinical evaluation of keratolytic activity of the combined formula showed highly significant induced keratolysis \((\text{Chi-Sq} = 8.025; \text{DF} = 1; \text{P-Value} = 0.005)\) in comparison with blank treated group. That was a clinical sign of improvement since keratolysis can convert closed comedos to opened type. Moreover, keratolysis insure more antiacne drug absorption fraction since it causes thinning of the corneocytes portioning. Different studies showed the importance of the use of keratolytics in treatment of acne\(^{25}\).

Redness is a major indicator of inflammation. It could be assessed objectively by RGB shift analysis. Redness reduction ratio was 2.5 at C.I. 0.95.

Test formula revealed a significant remission of impetiginization in comparison with blank treatment \((\text{Chi-Sq} = 4.531; \text{DF} = 1; \text{P-Value} = 0.033)\).

That effect may be attributed to the synergistic activity of levofloxacin and cloxacillin. Some studies on assessment of comedolytic effects of ciprofloxacin and ampicillin revealed significant influence of these drugs on improving acne\(^{26-29}\).

Conclusion

From the overall results, the combined retinol, levofloxacin, cloxacillin, ascorbic acid and the hydrocarbon base vehicle showed significant improvement in biometric outcomes of facial acne lesions. Larger sample size is necessary for further confirmation of the antiacne activity of the test formula. We recommend that other congeners of the used antimicrobials and keratolytics are to be included. And, for future studies, other comparative studies between antiacne drugs alone and in combination to determine the synergistic ratio.

Acknowledgement: A special gratitude and thanks is to the head of department of pharmacology-College of Medicine at University of Kufa for his help to use the lab. Thanks is to the technician in the department of pharmacology for his help in conducting the topical formulation.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References


Assessment the Efficacy of Arthrocentesis with Corticosteroid and Arthrocentesis with Sodium Hyaluronate in Treatment Temporomandibular Joint Disorders: A Comparative Study

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1University of Al-Ameed/College of dentistry-Iraq

Abstract

Background: Temporomandibular joint disorders (TMD) refer to a group of heterogeneous pain and dysfunction conditions involving the masticatory system, reducing life quality of the sufferers. Aim of study: The aim of the study was to evaluate the effectiveness of arthrocentesis with corticosteroid (betamethasone) injection and with sodium hyaluronate injection in treatment temporomandibular joint disorders, in conjunction with a stabilizing splint, for improving function and reducing pain, for preventing further deterioration of the TMJ dysfunction, to maintain improvements over time, and compare between them. Patients and methods: Fifty –four patients with TMJ disorders with age limit between 18 and 55 years of age, 9 males and 36 females, were enrolled in this study. Patients were randomly divided into two groups, in which one group received arthrocentesis with intra-articular corticosteroid (betamethasone) injection (1 ml), and another group received arthrocentesis with intra-articular sodium hyaluronate injection (1 ml) in superior joint space in single puncture. Patients were followed at regular interval of 7th day, one month, 3, 6, 9, 12 months (follow up) after last injection. Results: The mean age of patients was 8.873 ± 34.112 years in Group-A treat by betamethasone and10.973± 33.27 years in Group-A treat by sodium hyaluronate (ranged from 18.2 to 55.0 years). Keywords: Arthrocentesis, corticosteroid(betamethasone),Sodium hyaluronate, temporomandibular joint disorders

Introduction

Internal derangement of the temporomandibular joint (TMJ) is a progressive disorder which usually starts with clicking associated with normal mouth opening (anterior disc displacement with reduction),to a stage where clicking gradually ceases but restricted mouth opening ensues (closed lock). This was attributed to a nonreducible anteriorly displaced articular disc acting as an obstacle to the gliding condyle. In the meantime, the pathological changes were found in synovial membrane and synovial fluid. Hyaluronic acid is a principal component of the synovial fluid which plays an important role in nutrition, lubrication, anti-inflammation and cartilage protection and repairing. The synthesis, molecule weight, and concentration of hyaluronic acid are decreased during TMD and cause TMJ degenerative changes. TMD treatment can be divided into two categories: conservative method and surgical method. Among the surgical interventions, arthrocentesis is generally suggested for patients who are not responsive to conservative therapy.

Arthrocentesis is generally suggested for patients who are not responsive to conservative therapy. Arthrocentesis is an easy, minimally invasive, highly efficient procedure designed to decrease joint pain and increase the range of mouth opening in patients with closed lock of TMJ. This improvement in clinical outcomes after arthrocentesis can be attributed to the facts that the flow of liquid under pressure in joint causes flushing of catabolites, distension of joint with breakage of adhesions, and mobilization of disc.

Corticosteroids (CSs) are anti-inflammatory drugs that interrupt the inflammatory and immune pathways. They have been used for both therapeutic and diagnostic purposes. Also, they showed their palliating effects by suppressing inflammatory responses. Intra-articular corticosteroid injection alone or after arthrocentesis provides long-term palliative effects on subjective symptoms and clinical signs of TMJ pain.
Hyaluronic acid (HA) is a polysaccharide which is produced by chondrocytes and synoviocytes of the joints. HA has been shown to improve and restore normal lubrication in joint, provide nutrition to the avascular articulating disc, and stabilize the joint. The therapeutic mechanism of action of HA is chondroprotection, effect on proteoglycan and glycosaminoglycan synthesis, anti-inflammatory, mechanical (viscosupplementation), effect on subchondral bone, and analgesic2,9.

HA is a polysaccharide of low, medium, or high molecular weight, its properties can vary in relation to its molecular weight and shape, and has been used successfully as a TMJ injection to reduce inflammation, restore normal lubrication and cartilage repair2.

These have motivated us to perform the current study, to evaluate the effectiveness of arthrocentesis with corticosteroid (betamethasone) injection and with sodium hyaluronate (low molecular weight) injection in treatment temporomandibular joint disorders, in conjunction with a stabilizing splint, for improving function and reducing pain, for preventing further deterioration of the TMJ dysfunction, to maintain improvements over time, and compare between them during 12 months following injection.

Patients and Method

Fifty-four patients with TMJ disorders with age limit between 18 and 55 years of age, 9 males and 36 females, were enrolled in this study. All patients were examined clinically and radiographically. Based on the history and examination of patient a diagnosis of internal derangement was made. Patients were informed about the procedure, it is possible complication and about the material used and after the consent, patients were randomly divided into two groups, (27 in each group) and arthrocentesis was performed in each group following which 1 ml of betamethasone was given in first group and 1 ml of sodium hyaluronate in second group in superior joint space. Patients were followed at regular interval of 7th day, one month, 3, 6, 9, 12 months (follow up) after single injection. Assessment of clinical outcome was done in terms of reduction in pain (visual analog scale score), maximum mouth opening (MMO) in millimeters, painful/pain-free lateral or protrusive jaw movement, and clicking/crepitus of joint in pre-treatment visit about 1 week before injection and post-treatment follows up visits. This study was performed in Samir Dental Clinics in Karbala city, from October 2016 to Jun 2019. All patients were diagnosed with TMD based on Clinical finding supported by CT scan. In this study, selection of patients based on the following

Inclusion Criteria:

1- Clinical diagnosis of anterior disc displacement (Limitation of mouth opening, Pre-auricular pain, temporal and occipital tenderness, headache, Persistence of symptoms at least for 3 months, Clicking).

2- CT scan (soft tissue window depend on disk density) diagnosis of anterior disc displacement with reduction.

Each patient received pharmacotherapy and then if no or delay response splint fabricated then if no progression in treatment, arthrocentesis with intra-articular betamethasone injection and arthrocentesis with intra-articular sodium hyaluronate injection in superior joint space were done. Patients were informed of the use of their medical records. Ethical approval for the study was obtained from the ethical committee.

Also, we exclude other patients according to exclusion criteria: Systemic disease, Arthritis or history of condylar trauma, Degenerative change of condylar head, Facial asymmetry, retrognathism, prognathism, Fibromyalgia, use of NSAIDS within 48 hours, allergy to study medications, edentulous subjects pregnancy or breast feeding.

The statistical analysis was carried out using Statistical Package for Social Sciences (SPSS Inc). All quantitative variables were estimated using measures of central location (mean) and measures of dispersion (standard deviation). As data was normally distributed, paired t-test was applied for comparison of every two visits of each group. All statistical tests were two-sided and performed at a significance level of α= .05.

Results

The mean age of patients was 8.873±34.112 years in Group-A treat by betamethasone and10.973±33.27 years in Group-A treat by sodium hyaluronate (ranged from 18.2 to 55.0 years). A detailed sex and age distribution is shown in (Table-1). more reducing of mean and ±SD (standard deviation) values of the pain intensity, maximum mouth opening, joint click and deviation on opening were recorded in post-treatment visit at 12 month follow-up visit of Group-B(SH) than pre-treat visit, followed by Group-A(CS) is shown in (Table-2).
Inter study visits comparisons of each group regarding of the pain intensity, maximum mouth opening, deviation mouth opening and joint click revealed, Highly significant differences between pre-treatment visit and post-treat at 12 month follow-up visit after the single injection in both groups (Table-3) HS differences between post-treat at 12 month follow-up visit of Group -B(SH) and post-treat at 12 month follow-up visit of Group - A(CS) (Table -4).

Table 1: Sex and age distribution

<table>
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<tr>
<th>Group</th>
<th>Age</th>
<th>Gender</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>mean±SD</td>
<td>male</td>
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<tr>
<td>Group-A treat by betamethasone</td>
<td>8.873±34.112</td>
<td>5</td>
</tr>
<tr>
<td>Group-A treat by sodium hyaluronate</td>
<td>10.973±33.27</td>
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Table-2: Descriptive statistics of the pain intensity, maximum mouth opening, joint click and deviation on opening of mouth

<table>
<thead>
<tr>
<th>Clinical Parameter</th>
<th>Group A Pre-treat mean±SD</th>
<th>Group-A Post-treat at 12 month mean±SD</th>
<th>Group-B Pre-treat mean±SD</th>
<th>Group-B Post-treat at 12 month mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain intensity</td>
<td>7.27±0.273</td>
<td>1.85±0.260</td>
<td>7.77±0.381</td>
<td>0.36±0.231</td>
</tr>
<tr>
<td>Maximum mouth opening</td>
<td>35.16±0.259</td>
<td>41.02±0.281</td>
<td>35.48±0.411</td>
<td>43.67±0.227</td>
</tr>
<tr>
<td>Joint click</td>
<td>8.41±0.440</td>
<td>1.60±0.247</td>
<td>8.79±0.352</td>
<td>0.45±0.404</td>
</tr>
<tr>
<td>Deviation of mouth</td>
<td>7.77±0.278</td>
<td>1.94±0.249</td>
<td>8.38±1.571</td>
<td>0.41±0.345</td>
</tr>
</tbody>
</table>

Table-3: Comparisons between pre-treatment visit and follow-up visits of each group in the pain intensity, maximum mouth opening, joint click and deviation on opening of mouth

<table>
<thead>
<tr>
<th>Clinical Parameter</th>
<th>Group A Pre-treat vs. Post-treat at 12 month</th>
<th>Group B Pre-treat vs. Post-treat at 12 month</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T-test value</td>
<td>Df</td>
</tr>
<tr>
<td>Pain intensity</td>
<td>84.043</td>
<td>26</td>
</tr>
<tr>
<td>Maximum mouth opening</td>
<td>80.995</td>
<td>26</td>
</tr>
<tr>
<td>Joint click</td>
<td>62.479</td>
<td>26</td>
</tr>
<tr>
<td>Deviation of mouth</td>
<td>24.963</td>
<td>26</td>
</tr>
</tbody>
</table>

*Df: degree of freedom
Table -4: comparisons between post-treat at 12 month follow-up visits of each group in the pain intensity, maximum mouth opening, joint click and deviation on opening of mouth

<table>
<thead>
<tr>
<th>Clinical Parameter</th>
<th>Group-A Post-treat at 12 month vs. Group-B Post-treat at 12 month</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T-test value</td>
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<tr>
<td>Pain intensity</td>
<td>21.871</td>
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<tr>
<td>Maximum mouth opening</td>
<td>37.257</td>
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<td>Joint click</td>
<td>12.291</td>
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<tr>
<td>Deviation of mouth</td>
<td>18.209</td>
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</table>

**Discussion**

In the present study, clinical parameters such as pain, MMO, lateral and protrusive movement of jaws, and improved significantly in both the treatment arthrocentesis with intra-articular injection corticosteroid and arthrocentesis with Sodium hyalurate (SH). However, more significant improvement in pain, MMO, lateral and protrusive movement was observed in patients receiving arthrocentesis with intra-articular SH injection.

The outcome of the recent study is agree with systematic review of Eduardo et al. 2013, they were found that the effects of intra-articular injections with sodium hyalurate are similar to those regarding the injections with corticosteroids to control TMJ internal derangements at short and medium terms, while in long-term treatments, injections with sodium hyalurate showed better results.

The result of recent study is agree with study of Kapusuz G et al. 2014, that studied effectiveness intra-articular injections of hyaluronic acid, tenoxicam and betamethasone on the relief of temporomandibular joint disorder complaints, they found that HA produced better pain relief scores when compared to the other anti-inflammatory agents studied.

Radiological assessment preoperatively and postoperative 12 months follow-up with CBCT was showed significant difference. The erosion on condyles disappeared in the patients in both groups, it was significant, and showed radiological thin layer of new bone formation, remodeling of condyles and glenoid fossa in patients who treated by arthrocentesis with betamethasone, and cortical bone formation and remodeling of severe degenerative changes at 12months follow-up in patients who treated by arthrocentesis with sodium hyalurate, it is highly significant in patients receiving arthrocentesis with sodium hyalurate injection than significantly in patients receiving arthrocentesis with betamethasone.

The result of recent study is agree with study of Li et al. 2015, that studied changes of TMJ disorders in CBCT in patients who received HA injection in superior joint and reported cortical bone formation and remodeling of severe degenerative changes by 9 months follow-up.

This improvement in clinical outcomes after arthrocentesis can be attributed to the providing viscosupplementation to joints, HA has anti-inflammatory effects on inflammatory mediators, and protection against the disintegration of proteoglycans and cytotoxicity induced by oxygen free radicals. IL-1β is the key mediator in anti-inflammatory effects of HA and is regulated through HA-CD44 binding. IL-1β suppression results in downregulation of matrix metalloproteinases which also aids in anti-inflammatory effects of HA and further suppression of pro-inflammatory mediators IL-8, IL-6, prostaglandin E2, and TNF-α provides anti-inflammatory effects of intra-articular HA treatment. In addition, it affects leukocyte adhesion, proliferation, migration and phagocytosis; it directly influences the control mechanism of monocyte activation; in the cartilage it has been seen to suppress degradation of the cartilaginous matrix by fibronectin fragments.

Hyaluronic acid is found in the extracellular matrix of several connective tissues of high molecular weight, including joint cartilage and synovial fluid.
sites, HA molecules are predominantly synthesized. It is synthesized by synoviocytes, fibroblasts and chondrocytes present in the connective tissue. In addition, it is the largest natural component of SF and an important component of the articular cartilage. Moreover, activates intrinsic repair processes of the cartilage and normalizes the endogenous production of HA by the synoviocytes, stabilize the extracellular matrix, stimulate the proliferation of chondrocytes, regulate the production/degradation of type II collagen, and metabolic HA activity in cell renewal helps the nutrition of avascular zones of the disk and joint cartilage through its combination with glycosaminoglycans coming from proteoglycans produced by chondrocytes.

It is the major component of the synovial fluid and has an important role in lubrication. Its action stems from the ability of the polysaccharides to connect to each other when they are in solution, forming a network that provides a high degree of viscosity to the SF so that it reduce joint friction coefficient, that is the main risk factor for degenerative joint pathologies, maintaining intra-articular homeostasis by promote a better distribution of forces and load absorption of articular tissues. In cases of inflammatory and degenerative changes of joints, the concentration and molecular weight of HA acid reduced, therapeutical effect sodium hyaluronate increases the concentration and molecular weight of HA in the synovial fluid, restoring tissues lubrication and nutrition as well as minimizing mechanic stress. Moreover, intra-articular SH injection is avoided sensitization of pain receptors in joints disorders, by modulating neurotransmission and vasodilatation processes, provides an analgesic effect by blocking receptors and endogenous substances that cause pain in synovial tissues and. In addition, it promotes a release of adhesion areas between the articular disc and the mandibular fossa, increasing joint mobility and allowing better synovial fluid circulation.

Corticosteroids have a potent anti-inflammatory effect on synovial tissue and are known to reduce effusion, decrease pain and bring about an increase in range of motion of synovial joints.

Glucocorticoids have a very original mechanism of action, essentially genomic (transcriptional) and characterized by the activation (transactivation) or inhibition (transrepression) of numerous target genes. These molecules act in many cells, including not only innate immunity cells (macrophages, granulocytes, mast cells) and adaptive immunity cells (lymphocytes), but also other cells (fibroblasts, epithelial and endothelial cells).

Therefore arthrocentesis with sodium hyaluronate injection is more effectiveness than arthrocentesis with betamethasone injection in therapeutic and return TMJ of healthy status in long term palliative effects.

Conclusion

In this study, the technique of arthrocentesis using 0.9% normal saline solution with betamethasone injection, with occlusal splint in group A and arthrocentesis with intra-articular injection of sodium hyaluronate in superior joint space with occlusal splint wear in group B, where showed therapeutic benefits, simplicity, safety, patients acceptance of injection technique and lack of significant side effects and complication. Both techniques increased maximal mouth opening, lateral movements, and function, while reducing TMJ pain and noise. Although patients benefitted from both techniques, arthrocentesis with injection of SH is significantly superior to arthrocentesis with betamethasone injection. As well as, Radiological finding is showed highly significant in patients receiving arthrocentesis with HA injection than significantly in patients receiving arthrocentesis with corticosteroid.

Refreneses

5- Sato S, Sakamoto M, Kawamura H, Motegi K. Disc position and morphology in patients with


Aqueous Extract of Date Palm Fruit (*Phoenix dactylifera*) Protect Liver Against Cyproterone Acetate Toxicity in Male Mice

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¹University of Babylon, College of Science, Dept. of Biology, Iraq. ²Basic Science Department, College of Dentistry, University of Babylon, Hillah, Iraq

Abstract

The cyproterone acetate (CPA) is an antiandrogen drug that is used in the treatment of prostate cancer, which is related to drug-induced liver injury (DILI). The aim of this study is the effect of the water extract of one of the local dates on the side effects of using cyproterone acetate on liver tissue in white mice. Forty from albino mice male were divided into 8 equal groups received orally in one ml as follows. Group 1: distilled water passive control, group 2: corn oil positive control, group 3: received with 5 mg/kg body weight CPA, group 4: received with 20 mg/kg CPA, group 5: received with 5 mg/kg CPA & 60 mg/kg date palm extract, group 6: received with 5 mg/kg CPA & 120 mg/kg date palm extract, group 7: received with 20 mg/kg CPA & 60 mg/kg date palm extract, group 8: received with 20 mg/kg CPA & 120 mg/kg date palm extract lasted for 21 days.

Showed a histological study of the liver remarkable degeneration of hepatocytes associated with interstitial necrosis and blood vessel congestion.

The current study proved that the water extract of dates has a weak effect in the repair of damage in the liver tissue to treatment for the low dose of cyproterone acetate only.

Keywords: Cyproterone acetate, Date palm fruit, hepatotoxicity, histopathology.

Introduction

CPA is synthetic progesterone and antiandrogenic compound administered. It is used to treat many physical conditions in prostate cancer and also in breast cancer, serious acne, womanly hirsutism, precocious puberty, hypersexuality (1).

Drugs can have direct toxic effects (dose-dependent) or elicit hypersensitivity or metabolic distinctive reactions (dose-independent) that can take place at any time during the course of therapy (2). Hepatotoxicity signs resulting from the use of both steroidal androgens and nonsteroidal antiandrogens causes many cases as immunoallergic cytotoxic reactions, cholestasis, autoimmune hepatitis (3 & 4), acute hepatitis, fulminant hepatic fail (5) cirrhosis (6), and ultimately, CPA has been imputed a hepatocellular mutagenic capacity leading to hepato-carcinogenesis (7). Many treatments are for natural products can use because they are cheap and easy to obtain. The Prophet Mohammed (Peace Be upon Him) recommended the use of natural products as medicine for certain diseases (8). In traditional medicine, herbal medicines are widely used around the world, where palm pollen grains are widely used as an antihepatotoxicity (9 & 10).

*Phoenix dactylifera* L. plant is one of the elderly cultivated plants in the Middle East and North Africa. The fruits of dried dates contain 8 phenolic acids (gallic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, and ferulic acid (11) & (12). In addition, date fruits contain
Ascorbic acid, β-carotene, nicotinic acid, riboflavin and thiamine (13), and include twenty-one free amino acids (leucine, α-alanine, and proline were predominant), and the amides asparagine and glutamine were particular in P. dactylifera (14). Another study indicated that date palm fruit contains cholesterol, campesterol, stigmasterol, β-sitosterol, and fucosterol (15). Every date types are a user provenance of natural antioxidants It can be considered effective food (16) since date fruit extract had a powerful antioxidant and antimutagenic specialty (17).

Various studies have that the date fruit extract has been shown to ameliorate liver damage in rat, inhibit swelling and tumors, suppress the growth of Streptococcus pyogenes and improve sperm parameters (18) & (19).

**Materials and Method**

**Preparation of extract**

Fresh date palm fruits (*Phoenix dactylifera* L., *Palmae*) were provided from a local market in Hilla (Babil, Iraq), dried at room temperature, and were manually isolated from the pits. The flesh of the dried *P. dactylifera* fruits was ground. About 650 g of the powder was soaked in 2 L of cold distilled water. After 24 h, the solution was filtered and evaporated under vacuum and dried to a constant weight using a freeze-drier. The dry extract of the fruit was dissolved in distilled water instantaneously before treated the mice (20).

**Preparation of cyproterone acetate:**

Obtained cyproterone acetate anti-androgen from local pharmacies and called Androcur as a trading name and equipped from a company of a subsidiary of Filiale de Schering AG Germany, 20 mg concentration for each disc. Dissolved the drug used in this study in absolute ethyl alcohol and left exposed to the air until drought then added to the powder pure corn oil. Solute each disc in 12.5 ml from corn oil to obtained 20 mg/ml this study in absolute ethyl alcohol and left exposed to the air until drought then added corn oil and calculated required concentrations to conduct experiments depending on the dose given to human (21).

**Animals of the experiment:**

In the current study, 40 male white mice have used range ages from used 2-3 months. The mice were provided with eating and water *ad libitum*.

**Experimental protocols:**

Mice were randomly divided into eight groups each contains 5 animals treated daily with one milliliter orally as follows:

1-**First control group:** treated with distilled water for 21 days, as a negative control.

2-**Second control group:** treated with corn oil for 21 days, as a positive control.

3-**Third group:** treated with cyproterone acetate 5 mg/kg/BW for 21 days.

4-**Fourth group:** treated with cyproterone acetate 20 mg/kg/BW for 21 days.

5-**Fifth group:** treated with cyproterone acetate 5 mg/kg/BW and crude date extract 60 mg/kg/BW for 21 days.

6-**Sixth group:** treated with cyproterone acetate 5 mg/kg/BW and crude date extract 120 mg/kg/BW for 21 days.

7-**Seventh group:** treated with cyproterone acetate 20 mg/kg/BW and crude date extract 60 mg/kg/BW for 21 days.

8-**Eighth group:** treated with cyproterone acetate 20 mg/kg/BW and crude date extract 120 mg/kg/BW for 21 days.

Animals were sacrificed 24 hours after of the last dose, use diethyl ether to drugged mice, open the abdominal cavity and remove the liver, then fixed the fresh small pieces of each mouse liver in the formalin solution 10 % until the histological preparation.

**Histological study:**

Ordinary histological processing is prepared for the liver in order to study the histopathological changes that may be found in the experimental groups as compared with negative and positive control groups. According to (22), the processing steps and staining technique was as follow: small pieces of livers were dehydrated using a graded ethanol series, subsequently emb.edded in paraffin, wax blocks were cut by the microtome to prepare 5µm thick sections and stained with hematoxylin after deparaffinization of sections in xylene and hydrated in progressive descending ethanol series and stained with eosin after the washing and differentiate,
then wash again, dehydrate, cleared in xylene and mount with Canada balsam on glass slide for light microscopic examination.

**Results and Discussion**

Several studies have indicated in Liver enzyme aberration have in experimental animals treated with cyproterone acetate (23). This our study showed treated mice of 5 mg/kg body weight of CPA (group 3, fig. 1) revealed degeneration and necrotic changes in hepatocytes, sections of liver of CPA 20 mg/kg/ BW treated mice (group 4, fig. 2) showed congested blood vessels, necrosis in hepatocytes and moderate inflammatory cell infiltration in the portal triad as compared to other treated groups. The hepatotoxicity depend on both the dose and the duration of xenobiotics exposure will impact the type and grade of toxicity, there is often susceptibility to the toxicity based on the intralobular site of hepatocytes for xenobiotics that immediately affect the liver and hepatocellular necrosis, evidence of necrosis is generally apparent within forty-eight hour or previously(24).

One study noted an increase in serum aspartate aminotransferase (AST) and alanine aminotransferase. (ALT) activities in all patients with inprogress prostate cancer who are treated with CPA-induced liver damage, in 91% of those cases, the type of hepatic injury was hepatocellular damage. This damage is frequently involved hepatocytes damage that is associated with an elevated ALT level (25). It is generally synched progress that hepatocellular hypertrophy may be a serious qualitative metric, but classify the severity of hypertrophy is less accurate than relying on liver weight or quantitative measuration of enzyme induction. There is a substantial relationship between hepatotoxicity and enzyme induction with clinical pathology measurements are described (26). Changes may occur in liver histology without enzyme reduction but include fluid aggregation, fatty change in hepatocytes, inflammatory cell infiltration, fibrosis, and probably granuloma formation (27).

Examination of stained liver sections of animals treated with 5 mg/kg/BW of CPA and crude aqueous palm date fruit extract 60 mg/kg/BW (group 5, fig.3) showed a noticeable degeneration in hepatocytes, sections of the liver of CPA 5 mg/kg/BW and crude palm date fruit extract 120 mg/kg/BW treated mice (group 6, fig. 4) revealed congested blood vessels and necrosis of hepatocyte as compared with CPA treated mice with reducing severity in two groups. “The mechanism by which the aqueous date palm fruit extract induces its hepatoprotective activity versus oxidative damage caused by any drug is not clear. However, it is potential that polyphenolic compounds (flavonoids, anthocyanins, and phenolic acids), and trace elements (selenium, copper, zinc, and manganese), an extension to vitamin C present in the date palm fruit are the responsible compounds for this protection (28; 29; 30).

One of the studies conducted on the aqueous extract of date fruits It acts as an antioxidant and the antimutagenic activity, Where this extract on the inhibition of lipid peroxidation and protein oxidation and also by the aptitude to scavenge superoxide and hydroxyl radicals (31). In addition to that, there are many studies indicate the hepatoprotective activity of any drug is the ability of its components to block the aromatase activity of cytochrome P-450. On that basis, it is proposed that flavonoids in Phoenix dactylifera could be a factor contributing to its hepatoprotective ability through inhibition of cytochrome P-450 aromatase (32).

Tissue sections of the liver of CPA 20 mg/kg/ BW and palm date fruit extract 60 mg/kg/BW treated mice (group7, fig. 5) showed congested blood vessels and severe degeneration in hepatocyte, in the liver of animals treated with CPA 20 mg/kg/BW and palm date fruit extract 120 mg/kg/BW (group 8, fig. 6) Although comparative studies indicated that dried date palm fruit with phenolic content was higher than fresh date palm fruits(33) . However, the water extract of dried palm fruit used in this study did not have the ability to preserve the liver from the toxic effect of the drug CPA especially at a high dose. The direct effect of cyproterone is attributed to increased of hepatocytes of placental glutathione S-transferase, which are believed preneoplastic elements(34-35). Where it works Growth Factor-beta 1 (TGF) expression on the induction of apoptosis might account for both the liver damage and the expansion of liver tumors observed after giving a drug of CPA (36).
Figure-1: Histological liver section of (CPA) 5 mg/kg, group showed (a) Marked activation kupffere cell, (b) degeneration of the hepatocyte necrosis of hepatocyte.

Figure-2: Histological liver section of (CPA) 20 mg/kg group showed (a) sever degeneration of hepatocyte, (b) hypertrophied hepatocytes with deeply stained shrunken nuclei, (c) congested blood vessels.

Figure-3: Histological liver section of (CPA) 5 mg/kg + 60 mg/kg date palm extract, group showed (a) Some hepatocytes were free from nuclei and others contained pyknotic nuclei, (b) moderate hemorrhagic area.

Figure-4: Histological liver section of (CPA) 5 mg/kg + 120 mg/kg date palm extract, group showed (a) moderate hypertrophy of cells and (b) moderate hemorrhagic area.

Figure-5: Histological liver section of (CPA) 20 mg/kg + 60 mg/kg date palm extract, group showed sever degeneration of hepatocytes and multihemorrhagic Areas.

Figure-6: Histological liver section of (CPA) 20 mg/kg+120 mg/kg date palm extract, group showed (a) congested blood vessel (b) necrosis of hepatocyte (c) moderate inflammatory cells infiltration in the portal triad.

Conclusion

the present study has shown that CPA has a toxic effect and some histopathological changes have been detected, so care should be taken when CPA is prescribed as antiandrogenic treatment.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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References


Molecular Detection of Pseudomonas aeruginosa and its Relationship with Multidrug Resistance and Transposons

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¹Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Iraq.

Abstract

P. aeruginosa is currently one of the most frequently nosocomial pathogens and the infections due to this organism are often difficult to treat due to antibiotic resistance. P. aeruginosa is an important pathogen in hospitalized patient’s causative to their morbidity and mortality due to its multiple resistance mechanisms. Therefore, as a therapeutic option becomes restricted, the search for a new agent is a priority. One hundred and fifty samples were collected from different sources, divided into two main groups: clinical (80)samples and (70) hospital environment samples as a Nosocomial, collected all from October to the December of the year 2018. All of these samples were cultured by specific and differential media, Forty (40) isolates of P.aeruginosa bacteria were identified by using microscopic examination, biochemical tests. The identification of 40 isolates of P.aeruginosa bacteria confirmed by VITEK-2 system. A molecular detection the presence of Tnp-R gene in P. aeruginosa bacteria by conventional PCR to detect the Transposons and their relationship with multiple resistance of bacteria.

Keywords: Multidrug Resistance; P. aeruginosa; and PCR

Introduction

Pseudomonas aeruginosa is a Gram’s negative opportunistic pathogen has emerged as one of the most problematic of the nosocomial pathogens; considered multi-resistant infections in both community and hospital settings, It causes infections in cancer, burn, urinary tract, surgical wound, eye, blood, ear infection, sepsis cystic fibrosis, and (ICU) (1). Because of it’s an extremely ubiquitous organism and abundantly found in soil, water, plants, humans, animals, and in a hospital setting. P. aeruginosa is a common pathogen in hospital particularly in ICU although it has the ability to colonize healthy subjects, in addition to, bacterial exposure to some antibiotic classes may potentially induce endogenous resistance-conferring mutation in bacterial genes that encode drug targets (2). It has been progressively clear that resistance expansion in P. aeruginosa is their contexts with mutations in genes encoding porins, efflux pump, penicillin-binding proteins, and chromosomal β-lactamases, all contributing to resistance to β-lactamases, carbapenems, aminoglycoside, and quinolones(3). P. aeruginosa is an important pathogen in hospitalized patient’s causative to their morbidity and mortality due to its multiple resistance mechanisms. Therefore, as a therapeutic option becomes restricted, the search for a new agent is a priority (4). The pathogenicity of P. aeruginosa is largely caused by multiple bacterial virulence factors and genetic flexibility enabling it to survive in a varied environment. A number of these factors aid colonization, while others allow bacterial invasion(5). Antibiotic resistance in bacteria has reached a near-crisis point in nosocomial health care, with many bacterial isolates now multi-resistant as a result of the presence of additional DNA element. Earlier studies have shown that genes for resistance markers do occur on plasmids and they can be transferable, and most of them have demonstrated it by plasmid curing experiments alone. Resistance gene can occur on chromosomes, transferable plasmid, Transposons or jumping gene and specialized transposons called integrons that can assemble multiple resistance genes into the cassette (6, 7).

Materials and Method

- Specimens’ Collection:

During the period extended from October to the December of the year 2018, One hundred and fifty samples, divided into two main groups: (80)clinical samples and (70) samples hospital environment as a Nosocomial, were collected from hospitals. Clinical samples included: Urine samples from Urinary Tract Infections (UTI)patients, exudate samples from wounds
of the burn units patients, stool samples, Sputum samples from Cystic Fibrosis (CF) patients and Ear Swabs. The Nosocomial samples included many Nosocomial sources: Intensive Care Unit, Operations Hall, Birth Hall, Burning Hall, Devices and medical equipment, and hospital bed rooms.

- **Bacterial Isolation:**

In this study, the identification of 150 samples, we got 40 isolates of *P. aeruginosa* was performed by incubating these clinical and nosocomial isolates on different agar media (Nutrient agar , Blood agar, Maconkey agar, and Cetrime agar which are a selective media for *Pseudomonas spp.*) and the incubation at 37°C for 24 hrs. Forbes *et al.* (8).

- **Bacterial Identification:**

Identification of *P. aeruginosa* was confirmed by microscopically examination showed that it was single cells, a rod shape, not- spore-forming, and gram-negative, these results mention that this isolates may belong to *P. aeruginosa* growth on Cetrime agar for characterization of *P. aeruginosa* such as mucoid, smooth in shape with flat edges and elevated center, creamy green colour and have a fruity odour.

- **PCR amplification:**

DNA template of all isolates was prepared by boiling method (30 min in 100°C). The DNA of isolates was targeted for the *blaOXA-1* gene using the primers (Z.Tavajjohi, *et al.*, Iran) (9) listed in Table 1 and for the *Tnp-R* gene using the primers (Altaliby S. and Aldraghi w. , Iraq) and (Altaliby S. and Aldraghi w. , Iraq), and 8.5 μl of Nuclease Free Water. The experiment was continued according to the following program: initial denaturation at 94°C for 5 minutes, followed by 30 cycles at 94°C for 1 minute, 55°C for 1 minute, 72°C for 1 minute and a final extension at 72°C for 5 minutes. The PCR products were analyzed using gel electrophoresis (1% agarose) and stained with safe dye and visualized by Gel Doc apparatus (BioRad, USA) (Table 2).

### Table (1): Primers used in this study.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequence</th>
<th>Product size (bp)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>bla OXA-1</em></td>
<td>F 5'-AGCGTATAATTAAGCCC-3'</td>
<td>908</td>
<td>Z.Tavajjohi, <em>et al.</em>, 2011</td>
</tr>
<tr>
<td></td>
<td>R 5'-CTTGATTGAAGGGTTGGGCG-3'</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Tnp-R</em></td>
<td>F 5'-TTTTGTTATGCGCGGGTGC-3'</td>
<td>545</td>
<td>Altaliby S. and Aldraghi w. , 2018</td>
</tr>
<tr>
<td></td>
<td>R 5'-AGGCCCTTTCGTCTTCAAGA-3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table (2): Condition of PCR Reaction for *blaOXA-1* and *Tnp-R* genes of *P.aeruginosa*.

<table>
<thead>
<tr>
<th>Steps</th>
<th>Temperature</th>
<th>Time</th>
<th>Number of Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Denaturation</td>
<td>95°C</td>
<td>5 min.</td>
<td>1</td>
</tr>
<tr>
<td>Denaturation</td>
<td>95°C</td>
<td>30 Sec.</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>55°C</td>
<td>30Sec.</td>
<td></td>
</tr>
<tr>
<td>Extension</td>
<td>72 °C</td>
<td>30Sec.</td>
<td></td>
</tr>
<tr>
<td>Final extension</td>
<td>72 °C</td>
<td>7 min.</td>
<td>1</td>
</tr>
<tr>
<td>Hold</td>
<td>4 °C</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>
Results and Discussion

Isolation and Identification of *P. aeruginosa*:

One hundred and fifty samples clinical and Nosocomial samples were analyzed for the presence of *P. aeruginosa*, and the results of bacterial isolation and identification revealed the detection of forty (40) isolates of *P. aeruginosa*.

Biochemical Tests:

Some biochemical tests were performed for more validation. showed 40 isolates of *P. aeruginosa* provided by some biochemical tests, showed positive results for oxidase test, catalase test, motility test, , and production of *B*-hemolysis while (40) isolations negative results to citrate utilization tests, indole production and urease production tests negative to Gram’s stain and capable of growing on cetrimide agar as yellow greenish colonies (at 42°C for 24 hrs.). (Figure 1).

At the species level, *P. aeruginosa* has a wide growth temperature range, optimum growth at 37°C. Slower growth rates are seen at 4°C. *P. aeruginosa* is distinguishable from other clinically *Pseudomonas* spp. by its capability for growth at 42°C. They also grow well at pH range 6.6-7.0. It was tolerant of a wide variety of physical conditions, including temperature and pH\(^{10}\). Also, it was resistant to high concentrations of salts and dyes. It is typically given a positive result to the oxidase test and catalase. It does not ferment carbohydrates, but many strains oxidize glucose\(^{11}\).

The identification was performed with the automated VITEK -2 system using the GN-ID cards which contains 64 biochemical tests, from (40) isolate of *P. aeruginosa*, (40) positive result of the *P. aeruginosa* demonstrated.

**Distribution of *P. aeruginosa* according to Type of Specimens**

According to table (3), Out of (80) clinical samples of burns, sputum, urine, stool, ear and wound, 28(70%) isolates were positive to clinical *P. aeruginosa* and the percentage of the positive results from (70)Nosocomial samples were 12 (30%), as reported in (Table 4).

<table>
<thead>
<tr>
<th>Site of samples</th>
<th>Numbers of sample and Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>burn swab</td>
<td>2 (5 %)</td>
</tr>
<tr>
<td>wound swab</td>
<td>5 (12.5%)</td>
</tr>
<tr>
<td>ear swab</td>
<td>11 (27.5%)</td>
</tr>
<tr>
<td>Sputum</td>
<td>4 (10%)</td>
</tr>
<tr>
<td>Urine</td>
<td>6 (15%)</td>
</tr>
<tr>
<td>Total</td>
<td>28 (70%)</td>
</tr>
</tbody>
</table>

Figure (1): *P. aeruginosa* colonies on (A) Cetrimide agar, (B) and Blood agar, and (C) Nutrient agar after 24 hours of incubation at 37°C.
Table (4): Distribution of Pseudomonas aeruginosa isolates in Nosocomial samples

<table>
<thead>
<tr>
<th>Site of samples</th>
<th>Numbers of sample and Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICU</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Operations Hall</td>
<td>4 (10%)</td>
</tr>
<tr>
<td>Birth Hall</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Burn Hall</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Devices and medical equipment</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Total</td>
<td>12 (30%)</td>
</tr>
</tbody>
</table>

The low percentage was found in burn specimens which accomplished 5%. Results obtained reported that the highest percentage of P. aeruginosa from ear swab (27.5%) was in opposite with our results. In comparison with Nosocomial isolates of Pseudomonas aeruginosa the highest percentage isolation of Operations Hall (4%) where are other isolate reported low present in comparison with clinical findings.

P. aeruginosa is pathogenic only when introduced into areas devoid of normal defences, the bacterium attaches to, and colonizes the mucous membranes or skin, invades locally and produces systemic disease. Ps. aeruginosa infects healthy tissues rarely, but, when defences are compromised, it can infect virtually all tissues. This explains why most infections are nosocomial(12). These infections are Pneumonia, Osteomyelitis (related to Wounds, Immunocompromised patients, Burn-wound infections, Urinary tract infections, Endocarditis, external otitis and Tissue layer infections (13).

Genomic DNA Extraction:

Using a Genomic DNA Purification Kit (Promega), Genomic DNA was extracted from (40) P. aeruginosa isolates that were confirmed as bands by gel electrophoresis. Quantus Fluorometer was used to detect the concentration of extracted DNA in order to detect the goodness of samples for downstream applications. all the isolates had DNA concentration between (10-40 ng/μl) by Quantus Fluorometer.

Molecular Detection of P. aeruginosa and co-strains by blaOXA-1 like gene:

The result of PCR analysis concerning of the found the blaOXA-1 in (10) positive isolates, showed that studies P. aeruginosa possess the blaOXA-1 like gene from 10(25%) isolates positive showed 4 (40%) from clinical isolates and 6 (60%) from nosocomial.

The ESBLblaOXA-1 of P. aeruginosa isolates exhibited co-resistance against most of the antibiotics tested. This is consistent with most of the recent findings (14). The blaOXA-1 ESBLs provide P. aeruginosa with an additional powerful resistance mechanism with potentially serious clinical implications, including limitation of the therapeutic options. ESBLs manufacturing organisms create distinctive challenges to clinical microbiologists, clinicians, infection control professionals and scientists engaged in finding new antibacterial agents (15). The development and spread of ESBLs are most likely caused by the overuse of expanded- spectrum Ciprofloxacin in the hospital setting. Proper infection management practices and barriers are essential to stop spreading and outbreaks of ESBL-producing microorganism (16).

Molecular Detection of Transposons of P. aeruginosa:

The result of PCR analysis concerning of the found the Tnp-R gene in (2) (12%) positive isolates from 10 blaOXA-1 positive P. aeruginosa isolates, which identified the presence of Transposon in this bacteria as reported in figure(2).

Figure(2): Agarose gel electrophoresis (1% agarose, 100 Vol / mAm for 75 min.) of PCR amplification products (Tnp-R gene) at 545bp for P. aeruginosa. lane 30and 31 show positive results for P. aeruginosa; ladder 100 bp DNA marker.

Two strains of nosocomial isolates of P. aeruginosa exactly show bands for Tnp-R gene which represent the nosocomial sample number (30) and (31) were collected from the hospital’s operation hall and birth rooms respectively.
Strains of bacteria resistant to antibiotics, particularly those that are multi-drug resistant, are an increasingly major health care problem around the world. This is achieved through the cooperative activities of mobile genetic elements able to move within or between DNA molecules, which include insertion sequences, transposons, and gene cassettes/integrons, and those that are able to transfer between bacterial cells, such as plasmids and conjugal elements. Together these types of mobile genetic elements play a central role in facilitating horizontal genetic exchange and therefore promote the acquisition and spread of antibiotic resistance genes in both Gram-negative and Gram-positive bacteria, focusing on the group of organisms (S. aureus, K. pneumonia, A. baumannii, P. aeruginosa, Enterobacter spp., and Escherichia coli), which have become the most problematic hospital pathogens.

Whereas one or two classes are left in presence of Integron and/or Transposons, extensive drug resistance (XDR) MDR/XDR has appeared in P. aeruginosa, Acinetobacter baumannii, E. coli, and K. pneumonia, producing extended-spectrum β-lactamases (ESBL), vancomycin-resistant enterococci, Enterococcus faecium (VRE), MRSA, vancomycin-resistant Staphylococcus aureus VRSA, Salmonella enterica serovar Typhimurium, Shigella dysenteriae, and Burkholderia.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** Self-funding

**References**


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Antifungal Activity of Silver Nanoparticles Using *Penicillium Chrysogenum* Extract Against The Formation of Biofilm for *Candida Glabrata*

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¹College of Education for Pure Science / Ibn Al-Haitham, University of Baghdad/Iraq,
²College of Biotechnology / University of Al-Nahrain/Iraq

**Abstract**

The results showed that 71 isolates of *Candida* spp were isolated from patients with leukemia both women and men. Isolate 59 from *C. glabrata* while the number of isolates of *C. albicans*, *C. tropicalis*, *C. krusei* and *C. kefyer* were 6,3,2,1 respectively. The size of the nanoparticle was measured using AFM, The highest peak was 455 nm due to the presence of surface plasmons and another 243 nm wavelength, SEM showed the presence of particle of different sizes and distributed regularly and small silver nanoparticle. Effect of synergistic silver nanoparticle and antifungal agent (fuconazole) on the biofilm of *Candida glabrata*, capable of *C. glabrata* on adhesion of epithelial cells in the absence of silver nanoparticle and fluconazole, no adhesion between epithelial and yeast cells when adding silver nanoparticles, Decrease in surface adhesion between biofilm of the yeast and the epithelial cell when adding fuconazole, When collecting silver nanoparticles with fluconazole and adding it to epithelial cells exposed to *C. glabrata*, It led to the inability of the yeast to adhere to epithelial cells and then died. All experiments showed the least significant differences at 0.001 level.

**Key Words:** *Penicillium chrysogenum*, silver nanoparticle, *Candida glabrata*, antifungal.

**Introduction**

*Candida* yeast is transformed from a saprophytic organism into a pathogen due to *Candida*’s factors such as adhesion, protease production, phospholipids, hemolysin proteins, biofilm and germ tube formation, Pathogenesis also depend on the host’s immune system. One of the factors causing an increase in candidiasis is the chronic illness of people such as diabetes, weak immune system, malignant tumors, pregnancy and excessive use of antibiotics, which are factors for the emergence of infection. *C. glabrata* is a mono-chromosome group (haploid) that has no dimorphic form and severe opportunism in the genitourinary system and in the bloodstream Candidemia is particularly prevalent in older people and infected with HIV. *C. glabrata* is common in 15-20% of infections and many of its isolates are resistant to fungal antibiotics such as Amphotericin B and Fluconazole. *C. glabrata* is a mono-chromosome group (haploid) that has no dimorphic form and severe opportunism in the genitourinary system and in the bloodstream Candidemia is particularly prevalent in older people and infected with HIV. *C. glabrata* is common in 15-20% of infections and many of its isolates are resistant to fungal antibiotics such as Amphotericin B and Fluconazole. (5) indicated that yeast has the ability to form a biofilm which is environmentally important and helps them to survive as human pathogens by allowing them to escape host immunity mechanisms, resist antifungal and compete with other microorganisms. The formation of the biofilm is a key factor in species survival.

*Penicillium chrysogenum* is common in temperate and subtropical regions and is found in food products such as citrus and grains. (6) noted that *P. chrysogenum* was widely used in the industry and in the treatment of certain plant wastes and the production of enzymes such as Polyamine Oxidae and Phospho-glucanate dehydrogenase. It also has a high potential for production of Penicillin antibody and the first commercially produced penicillin.

(8) noted that fungi contains some distinctive advantages when used as biosynthesis for the production of nanoparticle compared to bacteria by producing larger

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amounts of Mechanism of action of silver nanoparticles (AgNPs) against yeast by targeting the biofilms of *Candida glabrata*, Analysis of the active electron microscopy revealed that the interaction between nano- Ag and *C. glabrata* cells during AgNPs exposure leads to changes in membranes which can be observed as holes on the surface of the membrane and lead to cell death(9). The technique of silver nanoparticles led to the movement of the drug into the tissues of the body, which was previously unreachable and was based on several factors including pH, temperature, solubility in the medicine, absorption of the surface-related drug and the spread of the drug through the matrix of nanoparticles(10).

**Materials and Method**

**Collections of Samples:** Collected 130 clinical samples taken from patients of leukemia from the City of Medicine/ Leukemia Department in Baghdad City

**Isolation of yeast:** Placing 100 microliters of blood on the sabroud dextrose agar (SDA)(11).

**Identification of *Candida***: For the purpose of diagnosing *Candida* was studied, Characterstions of Morphological(12), Purification of Colonies(13).

**Virulence of factor test:** The following experiments were performed, germ tube test (14). Biofilm formation test(15), Candida Chromgenic agar(16), the Vitek2 Compact System.

**Identification of *Penicillium chrysogenum***: The fungus of *Penicillium chrysogenum* according(17).

**Biomass of *Penicillium chrysogenum***: For the fungal biomass by(18).

**Preparation of silver nanoparticles in *Penicillium chrysogenum***: The silver nanoparticles were composed by observing in kind the color change of the yeast from the transparent color to the dark brown color(19).

**Characterization of nanoparticle using different microscopes:**

A. **Atomic Force Microscope**: Use this microscope to find out the size of nanoparticles and monitor the bio-processing of nanoparticles and know the particle size(20).

B. **UV-ViS Spectrophotometer**: The UV spectrometer is used to monitor the biotransformation of silver ions by means of UV spectroscopy of the reaction(21).

C. **Scanning Electron Microscope**: Use this microscope to determine the size and shape of the nanoparticles and to know the structural(20).

**Studying the synergistic effect of nanoparticle and antifungal for the biofilm agent of the *Candida glabrata***: This technique was used to test epithelial cells of the mouth on adhesion the biofilm to *Candida glabrata* (22) as follows:

A- First treatment: Take 0.5 ml of sediment containing epithelial cells (control treatment).

B-Second treatment: Take 0.5 mL of the sediment containing the epithelial cells and add 0.5 ml of *Candida glabrata*.

C- Third treatment: Take 0.5 ml of sediment containing epithelial cells and add 0.5 ml *Candida glabrata* and 50 microliters of silver nanoparticles composed with *Penicilium chrysogenum*.

D- Treatment 4: Take 0.5 ml of sediment containing epithelial cells and add 0.5 ml *Candida glabrata* and 100 mg of antifungal Fuconazole.

E- Treatment 5: Take 0.5 ml of sediment containing epithelial cells and add 0.5 ml *Candida glabrata* and add 100 mg of Fuconazole and add 50 microliters of silver nanoparticles with *Penicillium chrysogenum*.


**Results and Discussion**

**Distribution of infected patients with candidiasis:** The results showed that 71 isolates of *Candida* spp were isolated from patients with leukemia, both women and men, with 34 clinical cases of women. The 50-65 age group recorded 17 cases of 50% and 9 cases of the 20-30 years and 26.5%. The age group between 40-50 years recorded 5 cases and 14.7%. Finally, the age group of 17 years had the lowest rates of 8.8% and three clinical cases as in Table (1).
Table (1): Shows the distribution of women by age group Candidiasis patients due to leukemia.

<table>
<thead>
<tr>
<th>Age group</th>
<th>No. of infected women/34</th>
<th>%Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>50-65 years</td>
<td>17</td>
<td>50</td>
</tr>
<tr>
<td>40-50 years</td>
<td>9</td>
<td>26.5</td>
</tr>
<tr>
<td>20-30 years</td>
<td>5</td>
<td>14.7</td>
</tr>
<tr>
<td>Less than 17 years</td>
<td>3</td>
<td>8.8</td>
</tr>
<tr>
<td>Total</td>
<td>34</td>
<td>100%</td>
</tr>
</tbody>
</table>

Chi-Square ($\chi^2$)  
P-value          
---               13.594 ** 0.0036

** (P<0.01).

In the case of men, there were 37 clinical and positive cases of yeast Candida spp. The results showed positive results for yeast for leukemia patients in the age group 50-65 years in 14 cases and 37.9%, followed by age group 40-50 years and 11 cases and 29.7% The age group between 20-30 years, which was 7 cases and 18.9%. Finally, the lowest age group of 17 years recorded the lowest rates of 13.5% and 5 clinical cases table (2).

Table (2): shows the distribution of men by age group. Candidiasis patients due to leukemia

<table>
<thead>
<tr>
<th>Age group</th>
<th>No. of infected of Men/37</th>
<th>%Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>50-65 years</td>
<td>14</td>
<td>37.9%</td>
</tr>
<tr>
<td>40-50 years</td>
<td>11</td>
<td>29.7</td>
</tr>
<tr>
<td>20-30 years</td>
<td>7</td>
<td>18.9</td>
</tr>
<tr>
<td>Less than 17 years</td>
<td>5</td>
<td>13.5</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>100%</td>
</tr>
</tbody>
</table>

Chi-Square ($\chi^2$)  
P-value          
---               5.270 * 0.0530

* (P<0.05).

This is consistent with (24) which showed that Candidiasis is the fourth most common type of infection of the bloodstream and causes candidasis for patients in hospital. The increase in infection in these age groups is due to the availability of appropriate conditions such as immunodeficiency, long-term use of antibiotics and malignant tumors.

Identification of Candida spp.: Table (4), 71 isolates were obtained from clinical samples of women and men with leukemia, 59 C. glabrata from 71 isolates and 83.1%, while the number of isolates of C.albicans, C.tropicalis, C.krusei and C.kefyer were 6,3,2,1 respectively. These results were consistent with (25), indicating that C.glabrata was the second most common cause and 24% of Candida in the United States of America. In 2004 Candida glabrata was the main cause of Candidemia, and mortality rates for Candida glabrata patients were detected. 50% in cancer patients, and 100% in bone marrow patients.

Table (4): shows the distribution of Candida isolated from patients with leukemia from women and men.

<table>
<thead>
<tr>
<th>Candida spp</th>
<th>No. of Isolates of Leukemia/Women and Men</th>
<th>Percentage%</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.glabrata</td>
<td>59</td>
<td>83.1</td>
</tr>
<tr>
<td>C.albicans</td>
<td>6</td>
<td>8.5</td>
</tr>
<tr>
<td>C.tropicalis</td>
<td>3</td>
<td>4.2</td>
</tr>
<tr>
<td>C.krusei</td>
<td>2</td>
<td>2.8</td>
</tr>
<tr>
<td>C.kefyer</td>
<td>1</td>
<td>1.4</td>
</tr>
<tr>
<td>Total</td>
<td>71</td>
<td>100</td>
</tr>
</tbody>
</table>

Chi-Square ($\chi^2$)  
P-value          
---               177.662 ** 0.0001

** (P<0.01).

4. Biosynthesis of silver nanoparticles

Combining the biomass of Penicillium chrysogenum with the silver nitrate solution results in color difference. This indicates the formation of silver nanoparticle by the presence of surface plasmon, which is consistent with (26). The difference in color is due to the difference in the electron density of the nanoparticles that are different nano-size.

Characterization of silver nanoparticle.

Atomic Force Microscope (AFM) Results: The average square root value is equal to Root Mean Square = 2.12nm. The surface roughness of the membrane is
average Roughness = 1.54nm. This value is a proof of surface roughness. The particle size was found to be 18.83 nm. (27) showed that the nanoparticle were modified by Fusarium graminearum in different sizes and measured using AFM and began with a diameter of 1 nm.

UV spectrophotometer results:

The highest peak was 455 nm and another 243 nm wavelength and the highest peak due to the presence of surface plasmons either the second peak may indicate the presence of tyrosine and tryptophan residues found in the protein released from the yeast. This is explained(28) suggests that the reduction of silver nitrate to silver nanoparticles can be easily by using the UV spectrometer because silver nanoparticles can absorb light in the visible area due to Plasmon surface.

Scanning Electron Microscope (SEM) Results:

Fig. (1) showed the presence of spherical particle of different sizes and distributed regularly and small silver nanoparticles, this supports(29). That the surface of the plasmon reached a peak of 420 nanometers and that silver nanoparticles have a spherical shape.

Effect of synergistic silver nanoparticle of Penicillium chrysogenum and antifungal agent on the biofilm of Candida glabrata: Results showed the effect coefficients the synergy of silver nanoparticle particles For Penicillium Chrysogenum and antifungal agent against the Candida glabrata has different effects. Figure (5-A) shows the normal shape of the epithelial cells that represent the control treatment, between Figure (5-B) capable of C.gabrata on adhesion of epithelial cells in the absence of silver nitrate and antifungal. Figure (5-C) showed no adhesion between epithelial and yeast cells when adding silver nanoparticles with Penicillium chrysogenum. This indicates the inability of C.glabrata to adhere to the presence of silver nanoparticles. The exposure of epithelial cells with C.glabrata and antifungal fluconazole showed a decrease in surface adhesion between biofilm of the yeast and the epithelial cell as shown in Figure (5-D). When collecting silver nanoparticles with fluconazole and adding it to epithelial cells exposed to C.glabrata, Yeast inability was observed on adhesion with epithelial cell and then its death due to the presence of synergistic nanoparticle with fluconazole showed stable and strong antifungal activity as in Fig. (5-E). The results showed that silver nanoparticle have properties antifungal.It can also provide synergy with antifungal when evaluating the synergistic effect of silver nanoparticles and the fluconazole against the adhesion cells formed for the biofilm of C.glabrata, This is consistent with the study of silver nanoparticle antifungal such as floconazole against Candida albicans and a strong synergistic effect between silver nanoparticles and antifungal(30).

(31) showed that silver nanoparticles are associated with important cellular structures of proteins and DNA and cause cellular damage to yeast. (32) explain The association of the silver atoms with the thiol group (SH) in the enzymes, which change the composition and function of the enzymes in the cell membrane, which makes the adhesion ineffective.
Figure (2) A- Normal epithelial cells, B- Epithelial cells with C. glabrata, C- Epithelial cells with C. glabrata when adding silver nanoparticles, D- Epithelial cells with C. glabrata when adding Fluconazole, E- Epithelial cells with C. glabrata when adding silver nanoparticles with Fluconazole.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References


Study of Epidemiological Factors According to the Positive Response of IgG of Patients Infected with *Blastocystis hominis* in Diyala province, Iraq

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**Abstract**

**Background:** *Blastocystis hominis* (*B.hominis*) is the most common intestinal parasite in humans and many other animals. Infections with the organism are spread worldwide and some of them have been asymptomatic, acute symptomatic and chronic.

**Materials and Method:** 100 blood samples were collected from patients with *B.hominis* which were reviewing to some Hospital and health center in Diyala province during the period from October 2018 to March 2019. To determine the extent of IgG antibody responses in serum patients infected with *B.hominis* for depending on the chromatic changes resulting from the association of antigens with antibodies.

**Results:** The results of the current study showed the percentage of infection among males was (56.25%), which is higher than that of females (43.75%), and the age group (3-6) years among males showed the highest rate of infection (33.34%) and the lowest in age groups less from one year and (9-12) years groups at (11.11%). The rate of infection in the age groups (1-3) years and (6-9) years was (22.22%) and in the age group of (1-3) years was the highest incidence among female groups (42.86%), while the age group (3-6) years showed less than that (28.57%) and significant differences at the level of probability less than (0.05). The percentage of *B. hominis* infection increased among the rural population by (56.5%), male infected, (57.14%) female, while the proportion of males among the urban population was (44.44%) and females reached (42.86%), with a standard deviation of (1.600 ± 0.495) and (1.480 ±0.505) respectively.

**Conclusions:** The presence of immunoglobulin IgG in serum patients has been shown to stimulate the cellular immune response and be indicative of long-term immunity against pathogenic antigens.

**Keywords:** *Blastocystis hominis*, diagnosis, parasite, infection, patients.

**Introduction**

*B. hominis* is one of the most common protozoa intestinal parasitic diseases worldwide and a common infected among humans and animals[1]. It isolated from stool specimens appear as unicellul and it has multiple shapes, such as vacuolar, granular, and amoeboid [2]. It was considered as harmless yeast, but it is now getting acceptance as an agent of human intestinal disease especially under immunosuppressive conditions[3]. The extent research on *B. hominis* is transmission mechanisms, incubation period, epidemiology, and treatment options[4]. It’s status as a true pathogen is controversial - while it has been found in patients with gastrointestinal symptoms with diarrhea or severe abdominal pain. Symptoms associated with human papillomavirus infection include: diarrhea, nausea, colic, abdominal distension, fever and chills. It is not proven to be the cause and many carriers are asymptomatic[5].
Diagnosis of B. hominis is used by clinical diagnosis of diarrheal symptoms and dehydration of the patient and laboratory methods. Direct microscopy of stool and cystic phase observation overlap with other causatives of diarrhea specially[6,7]. These data indicate that B. hominis induces as well as modulates the immune response in intestinal epithelial cells, and we conclude that different pathophysiological events may occur during B. hominis infection. [8]. The spread of disease is associated with poor hygiene, exposure to animals and the consumption of contaminated food or water [9].

Iraq has seen widespread outbreaks of gastrointestinal diseases. The Iraqi Ministry of Health and contributed many factors in the spread of the disease, including displacement of people to other places and water pollution. There are several studies that showed prevalence in different Iraqi governorates of the country. The rate of infection was recorded in 2013 (5.08%) for (31) children in Dohook in northern Iraq suffers from gastrointestinal symptoms[10]. 28 (4.1%) was infected with B. hominis from a total of 861 reviewers [11]. While in Muthana province in southern Iraq, the infection rate was 58 (45.67%) out of 127 patients infected with B. Hominis [12]. The aim of the present study is to investigate the most important immunological changes associated with infection in patients with B. hominis, the study has included measuring levels of certain cellular dynamics in the serum of infected by and based on the principle of color change resulting from the correlation of quality IgG antibodies and measured color change resulting mediated.

Statistical Analysis

Statistical analysis of the results of the current study using the Statistical Package for Social Sciences conducted (SPSS) metadata. Test was used T-test, variance analysis ANOVA, Chi-square, percentage, standard deviation and standard error in the present study to find a moral differences between groups with infection Blastocytis hominis and there were significant differences (P < 0.05).

Results

The present study included 100 patients with B. hominis, 50 males and 50 females. The positive response rate of IgG for males was higher than that of females, which recorded positive response rate of (56.25%) at (1.820±0.388). While the percentage of response to the antibiotic in the serum infected females (43.75%) at (1.860±0.351). Hence, the statistical analysis of this increase indicates that there isn’t a significant difference of statistical significance at the level the probability is less than 0.05 as shown in Table 1.

Materials and Method

Collection of samples

The present study collected 100 cases with Blastocystis hominis within the period from October 2018 to March 2019, of 50 male and 50 female, ranged in age less one year to 12 years. It has diagnosed the disease process and determined by dermatologists at some Hospital and health center in Diyala province and the injury was diagnosed based on the clinical symptoms and distinct phenotypic traits of infections.

Serological diagnosis

Serological diagnosis was performed by measuring the IgG level in the serum of patients with B. hominis to determine the response of the positive in the body of the infected patients. Blood samples were collected from patients. Was withdrawn 5 ml of venous blood for each of the infected persons, therapists and control the use of a syringe, put blood samples in test tubes and let the blood to clot at room temperature (20-25) minutes and then the separation of the serum Centrifuge device speeds of (3000) rpm and then it is kept freeze-preserved models when (-20°C) until the subsequent immunological tests.
Table: Effect of the sex factor on the positive serotonin of the IgG antibody of *Blastocyte hominis* for children covered in the infection

<table>
<thead>
<tr>
<th>Groups</th>
<th>number</th>
<th>Positive response</th>
<th>Percentage%</th>
<th>Mean±SD</th>
<th>Stender Error(SE)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys</td>
<td>50</td>
<td>9</td>
<td>56.25</td>
<td>1.820±0.388</td>
<td>0.055</td>
<td></td>
</tr>
<tr>
<td>Girls</td>
<td>50</td>
<td>7</td>
<td>43.75</td>
<td>1.860±0.351</td>
<td>0.049</td>
<td>0.280</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>16</td>
<td>32%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2 shows that the injuries spread within a wide range of age groups. The age of those infected was less than 1 to 12 years. In males recorded age groups the (3-6) year age group had the highest IgG response rate at (33.34%) and followed by tow the age groups (1-3) years and (6-9) years (22.22%) and less than at (11.11%) in tow groups less one years and (9-12) years age groups. Compared to the female group, the (1-3) years age group had the highest incidence of IgG infection and response 42.86%. The lowest response among the age group of females was 0.00% in the age group less than one year, so that no percentage is recorded. This is due to the visitors who visit the health centers at the time of collecting samples. While the age group 3-6 years by 28.57% and less than the age groups (6-9) and (9-12) years by (14.29%). Differences between the proportions of m and their response to IgG were statistically significant at the probabilistic level the level the probability is less than 0.05 as shown in Table 2.

**Table 2: Distribution of numbers and percentages of infection of *Blastocystis hominis* for the age of the infected and the extent of IgG response**

<table>
<thead>
<tr>
<th>Age groups in years</th>
<th>Number</th>
<th>% Number positive response</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Boys</td>
<td>Grils</td>
<td>Boys</td>
</tr>
<tr>
<td>&lt;1-</td>
<td>5</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>1-3</td>
<td>4</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>3-6</td>
<td>7</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>6-9</td>
<td>12</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>9-12</td>
<td>22</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>50</td>
<td>9</td>
</tr>
</tbody>
</table>

The patients’ place of residence showed a clear difference between their urban and rural residence, but did not constitute a significant difference of statistical significance at the probability of p> 0.05. The prevalence of IgG immunoglobulin was significantly higher among urban than in rural areas as table3 showed. The proportion of males was 55.56% of respondents 5 patients of the total number is 20 cases, while females registered 57.143%, with 4 patients of the total number of 26 cases. The response rate in the urban group of infected males was 44.44% with 4 patients of 30 cases, less than 42.857% in urban areas, with 3 patients of the total 24 cases.
Table 3: Effect of the living factor on the positive serotonin of the IgG antibody of Blastocystis hominis for children covered in the infection

<table>
<thead>
<tr>
<th>Residence</th>
<th>Number tested</th>
<th>Number positive</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Boys</td>
<td>Girls</td>
<td>Boys</td>
</tr>
<tr>
<td>Rural</td>
<td>20</td>
<td>26</td>
<td>5</td>
</tr>
<tr>
<td>Urban</td>
<td>30</td>
<td>24</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>50</td>
<td>9</td>
</tr>
<tr>
<td>mean±SD</td>
<td>1.600±0.495</td>
<td>1.480±0.505</td>
<td></td>
</tr>
<tr>
<td>Stander Error(SE)</td>
<td>0.699</td>
<td>0.714</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

B. Hominis is a protozoan intestinal parasite disease that caused by the emergence of various symptoms, including diarrhea, nausea, abdominal cramps, bloating, excessive gas, and anal itching. The timescale of infection with the parasite can range from weeks to years [13]. The present study showed an increase in IgG and positive response rate in males, with (56.25%) infection rate and (43.75%) female infection as in the table (2). This may be due to the number of hospital-reviewed cases and some male health centers more than females, and therefore dependent on individual immunity. This is consistent with the findings of Nayef et al. (2011) which indicated the percentage of male (23.99%) height on females (16.27%) and attributed the reasons for this to the above, attributed the reasons to as exposure to the infection increases with the top of nutrition and increased funding [14]. The percentage of infection between females and males does not make any significant difference at the probability level (0.05), which is consistent with the results of Mahmood and Khudher (2016) Indicating that there is no significant difference between the rates of infection of males and females and accounted for the percentage of infection B. hominis in Sammara city - Salah Al-Deen province was (9.09%) [15].

The current study indicates that the housing factor has a difference in the percentage of IgG positive response for patients with B. hominis for both sexes but does not register any significant difference at the probability level (P > 0.05). The positive response rate for patients with B. hominis who live in rural areas is higher than that of urban dwellers. This is due to the pollution of the environment and the environment of the rural inhabitants of B. hominis. They may be directly related to the reservoir stocks such as sheep, cattle, cats, dogs and other domestic animals. For rural males (55.56%) of respondents (5) patients of the total number is (20) cases, while females registered (57.143%), with (4) patients of the total number of (26) cases. The response rate in the urban group of infected males was (44.44%) between different age groups ranging from 6 months to over 61 years out of a total of (177) patients with B. hominis [16]. The main cause of diarrhea is B. Hominis among other pathogens, as explained by al-Kaissi and Majdi (2009), the highest proportion of whom were 82 cases with B. Hominis isolated from 200 patients suffering from diarrhea by 41% [17]. Where the current study recorded the age group (3 – 9) years is the highest response rate positive for IgG among males infected with B. hominis, which amounted to (33.34 %) and the lowest was in the two age groups less than one year and (9-12) years is (11.11%). While the age group (1-3) years among females infected with the highest rate of (42.86%) and less than in the categories of age (6-9) years and (9-12) years is (14.29%), while the category less than a year does not register any case among females and this is due to patients reviewing hospitals and centers when collecting samples as showed in table (2) .

The current study indicates that the effect of the age group in the incidence of B. hominis, whose age ranged between (> 1 - 12 ) years with a significant difference of (P < 0.05). As it was the highest rate of infection in both genus and is attributed to the lack of development of the immune system in the younger age groups compared to the large age and this is consistent with what Salman (2015), who pointed to a significant difference at level (P <0.05)
with (4) patients of (30) cases, less than (42.857%) in urban areas, with (3) patients of the total (24) cases as showed table (3).

Conclusions

The present study conclude that the disease’s ability to stimulate the cellular immune response leading to the production of IgG that observed height in patients groups.

Conflict of Interest: None

Source of Funding: There is no funded support for the implementation of this work, while the active role of us as joint researchers in the design of the study and the collection of samples and analysis and preparation of the manuscript and financial and moral support.

Ethical Clearance: Blood sampling was performed from October 2018 to March 2019 with the consent of the reviewing patients to participate in this study. This study was completed.

References


Epidemiological and Histopathological Study of Appendicitis in Karbala Province

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Abstract

The current study included an epidemiological prevalence study of appendicitis in Karbala province, where complete data were collected for the year 2018 from Al Hussein medical Hospital. The results showed that the total number of appendicitis in Karbala province was (636) cases, divided in to (299) and (286) for male and female respectively, while the secondary removal cases was (69). The percentage of infection was 47.012%, 42.12% and 10.85% for male, female and secondary removal. The statistical analysis showed that there were significant differences under (P> 0.05) between percentage of infection in male and female (0.01). Also this study included histopathological examination of appendectomy specimen. After surgical removal of appendix, the specimen directly fixed with 10% formalin and then do tissue processing. The microscopic examination showed obstruction of the lumen and infiltration of inflammatory cell within lumina properia, muscularis and adventitata. the mucosal epithelial was destruction with ulceration, also there are extensive lymphoid hyperplasia.

Keywords: appendicitis , prevalence, Karbala, gender, histopathological.

Introduction

Appendicitis is inflammation of the vermiform appendix, this is a hollow organ located at the tip of the Cecum, usually in the right lower quadrant of the abdomen, however it can be located in almost any area of the abdomen depending on if there were any abnormal developmental issues (situs inversus totalis) or if there are any other concomitant conditions such as pregnancy or prior surgeries (1).

The appendix develops embryonically at the fifth week, during this time there is a movement of the midgut to the external umbilical cord with the eventual return to the abdomen and rotation of the Cecum, this results in the usual retrocecal location of the appendix (2).

Appendicitis is more common surgical emergencies, and it is one of the most common causes of abdominal pain (3). The exact function of the appendix has been a debated topic, today it is accepted that this organ may have an immunoprotective function and acts as a lymphoid organ especially in the younger person, other theories contend that the appendix acts as a storage vessel for “good” colonic bacteria, still others argue that it is a mear developmental remnant and has no real function (4).

Appendicitis is most common between the ages of 5 and 40, the median age is 28, risk factors include being male, higher household income and living in a rural area (5).

In the United States, there were nearly 293,000 hospitalizations involving appendicitis in 2010. Appendicitis is one of the most frequent diagnoses for emergency department visits resulting in hospitalization among children ages 5–17 years in the United States (6).
Research aims:

1-study the epidemiological prevalence of appendicitis in Karbala province.

2-study the histopathological changes occur in appendix tissues during acute appendicitis.

Research methodology and procedures:

A database of patients with appendicitis was collected from Al Hussein Medical City in the Karbala city from January to December 2018. Also, samples were taken from the surgical theaters after being fixed directly with 10% formalin for tissue processing.

Results

1- Epidemiological study

All samples that collected in this study were under different condition, like; gender and age. The total number of appendicitis that show in this study in Karbala province was (636) cases divided into (299) and (268) for male and female respectively, while the secondary removal cases was (69). The percentage of infection was 47.012%, 42.12% and 10.85% for male, female and secondary removal, respectively. The statistical analysis showed that there were significant differences under (P > 0.05) between percentage of infection in male and female (0.01). Table (1).

Table (1): Percentage of appendicitis according to gender:

<table>
<thead>
<tr>
<th>Gender</th>
<th>Cases number</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>299</td>
<td>47.012%</td>
</tr>
<tr>
<td>Female</td>
<td>268</td>
<td>42.12%</td>
</tr>
<tr>
<td>Secondary removal</td>
<td>69</td>
<td>10.85%</td>
</tr>
<tr>
<td>Total</td>
<td>636</td>
<td>100%</td>
</tr>
<tr>
<td>Chi-Square</td>
<td>---</td>
<td>9.261 **</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td>0.0001</td>
</tr>
<tr>
<td>** (P&lt;0.01 ).</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This study also show that the highly percentage of infection in both male and female was in age between 10-20 years (35.82% in female and 42.47% in male), while the low percentage was in age between 60-70 years (1.11% in female and 0.66% in male) Table (2) and Table (3).

Table (2): percentage of appendicitis according to Female age:

<table>
<thead>
<tr>
<th>Age / Female</th>
<th>Cases number</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 10 year</td>
<td>32</td>
<td>11.94%</td>
</tr>
<tr>
<td>&lt; 10 ≥ 20 year</td>
<td>96</td>
<td>35.82%</td>
</tr>
<tr>
<td>&lt; 20 ≥ 30 year</td>
<td>81</td>
<td>30.22%</td>
</tr>
<tr>
<td>&lt; 30 ≥ 40 year</td>
<td>40</td>
<td>14.92%</td>
</tr>
<tr>
<td>&lt; 40 ≥ 50 year</td>
<td>9</td>
<td>3.35%</td>
</tr>
<tr>
<td>&lt; 50 ≥ 60 year</td>
<td>7</td>
<td>2.61%</td>
</tr>
<tr>
<td>&lt; 60 ≥ 70 year</td>
<td>3</td>
<td>1.11%</td>
</tr>
<tr>
<td>Total</td>
<td>268</td>
<td>100%</td>
</tr>
<tr>
<td>Chi-Square</td>
<td>---</td>
<td>10.026 **</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td>0.0001</td>
</tr>
<tr>
<td>** (P&lt;0.01 ).</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table (3): percentage of appendicitis according to Male age:

<table>
<thead>
<tr>
<th>Age / Male</th>
<th>Cases number</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 10 year</td>
<td>32</td>
<td>10,7%</td>
</tr>
<tr>
<td>&lt; 10 ≥ 20 year</td>
<td>127</td>
<td>42,47%</td>
</tr>
<tr>
<td>&lt; 20 ≥ 30 year</td>
<td>90</td>
<td>30,10%</td>
</tr>
<tr>
<td>&lt; 30 ≥ 40 year</td>
<td>37</td>
<td>12,37%</td>
</tr>
<tr>
<td>&lt; 40 ≥ 50 year</td>
<td>7</td>
<td>2,34%</td>
</tr>
<tr>
<td>&lt; 50 ≥ 60 year</td>
<td>4</td>
<td>1,33%</td>
</tr>
<tr>
<td>&lt; 60 ≥ 70 year</td>
<td>2</td>
<td>0,66%</td>
</tr>
<tr>
<td>Total</td>
<td>299</td>
<td>100%</td>
</tr>
</tbody>
</table>

Chi-Square 10.934 **
P-value 0.0001

** (P<0.01).

2- Histopathological study:

In present study the histopathological changes observed in appendix were presence of extravascular polymorphs in the epithelium, lamina propria, or muscular layers was the main diagnostic feature of acute inflammation (Figure 1 B, C, D). The wall of appendix, was clearly visible and the mucosa was largely destroyed (figure 1(A, and D)), and there was extensive neutrophil infiltrate extending throughout the submucosa and into the muscularis externa. The was seen in all specimens. The glands of appendix was largely affected, and shows the mucosa glands was destroyed and pus present at the base of the gland and with only few remnant of glands in the section (Fig.21 B).

Figure (1) A: show lumen obstruction and mucosa destruction of appendix.  
Figure (1) B: Show the lymphoid hyperplasia
Discussion of Results

Our study was revealed increase incidence of appendicitis between males compare with females, that may be related with female sex hormones, which has been proposed because of lower incidence among women and incidence variations during the menstrual cycle \(^7\).

Anderson was found inverse relation between pregnancy and appendicitis, this suggests that pregnancy protects against appendicitis, especially in the third trimester. During pregnancy a range of physiological changes take place that may influence the pathogenesis of appendicitis \(^8\). Our finding was agree with \(^9\) who reported increase of incidence of appendicitis in males, this due to variation in body physiology between male to female.

In our study the highly percentage of infection is rang between age (10-20) years, that cited by \(^10\), who reported that incidence of appendicitis is generally a disease of young age. \(^11\) was reported in his study the incidence outcome of appendicitis are related to age in young people. It has been suggested that the peak in the development of lymphoid tissue which occurs during adolescence leads to an increased liability of the appendix to obstruct, and so accounts for the high incidence of the disease.

the present histopathological study showed lymphoid hyperplasia with increase in lymph nodules diameter, these lead to obstruction. Our study was revealed increase in wall thickness of appendix and narrow lumen, with increase infiltration of white blood cells was very visible, this finding agree with \(^12\) and \(^13\) they recorded infiltrate of neutrophile in the muscularis mucosa and sub mucosa.

Obstruction of the appendiceal lumen seems to be essential for development of appendiceal infection. This obstruction occurs due to mucosal inflammation and lymphoid hyperplasia, once obstruction occurs, continued mucus secretion and increase intraluminal pressure, which obstructs lymphatic drainage and edema, mucosal ulceration and may cause venous obstruction, finally ischaemic necrosis of appendix wall produces gangrenous appendicitis.

Conclusions

1- Appendicitis is one of emergency disease.

2-The total cases in Karbala province was 636 in 2018 year which is divided to (299) and (286) for male and female respectively, while the secondary removal cases was (69).

3- The percentage of infection in male is higher than in female 47.012%, 42.12% and 10.85% for male, female and secondary removal.
4- Highly percentage of infection in both male and female was in age between 10-20 years (35,82% in female and 42,47% in male), while the low percentage was in age between 60-70 years (1,11% in female and 0,66% in male).

5- The most histopathological lesions that found in appendectomy specimen was lumen obstruction and mucosa destruction of appendix, lymphoid hyperplasia, obstruction of the lumen and infiltration of inflammatory cell within lumina properia, muscularis and adventitia.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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**References**


Molecular investigation of virulence factors genes in *streptococcus pyogenes* by PCR

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**Abstract**

This study was designed to detect some genes associated with important virulence factors in *streptococcus pyogenes*. A total of 200 throat swabs were collected from patients suffering from pharyngitis from both sex and age from (1 - 15) years. carried out from ENT unit in Al-Hilla General Teaching Hospital and Al Noor Hospital during the period from January 2018 to December 2018. Out of the 200 samples only 177 samples showed positive bacterial culture. No growth was seen in other 23 samples. The results indicate that the rate of *Streptococcus pyogenes* isolated from patient with pharyngitis is 30 (15%), others bacterial growth 147 (73%), and no growth 23 (12%).

Molecular detection of virulence factor genes was done like M protein (*emm*) gene, the result shows that these genes were detected in all isolates bacteria (100%) with molecular length (914 bp), *SpeA* gene was carried by using specific primer and it was found that (3.3%) isolates give positive result for this gene with amplicon (576 bp), *SpeB* gene the result shows that (100%) isolates contain the gene with molecular length (952 bp), *SpeC* gene also study by using specific primer at molecular length (405 bp) and the result shows that (75%) give positive results to this gene, the *mac* gene in all *S.pyogenes* isolates results show that (30%) give positive, results to this gene with molecular length (389 bp), *scpA* gene in all isolates the result shows that 17 isolates (56.6%) give positive result to this gene with amplicon size (622 bp)

**Keywords:** PCR ; *streptococcus pyogenes* ; virulence factors

**Introduction**

*Streptococcus pyogenes*, commonly known as group A streptococcus (GAS) is a fermentative, facultative anaerobe, nonmotile, nonspore-forming gram-positive coccus, which occurs in chains or pairs, having a diameter of 0.5-1.0 μm. GAS are beta haemolytic streptococci. They require an enriched medium containing blood to grow (1). The group A streptococci are fastidious organisms that have complex growth requirements. A highly nutritious growth medium that provides optimal growth GAS is generally grown on agar media supplemented with blood (2). Pharyngitis, or commonly known as sore throat or strep throat, is the most common manifestation of infection with *Streptococcus pyogenes* (GAS) Infection with this bacterium is diagnosed in 20 to 40% of pharyngitis cases in children and in 5 to 15% in adults (3). M protein considered one of the most important virulence factors, the M protein promotes host interactions and adherence to human epithelial cells; specially helping bacteria to escape from host immune response by inhibition of phagocytosis (4). Other important virulence factors include the streptococcal superantigens (SAgs). SAgs are bacterial toxins which bind to major histocompatibility complex class II and T-cell receptors (5).

*SpeB* plays a role in the pathogenesis of *S. pyogenes* infections by Destruction of host defense system proteins and cleavage of GAS surface proteins may help the bacterium to escape immune clearance, invade the deeper tissues, and disseminate from the primary infection site (6). *SPE-C* was the first streptococcal SAg SPE-C binds to the polymorphic MHC class II β-chain with the formation of a tetravalent zinc complex that includes three residues within the C-terminal domain of SPE-C (7). Mac-2 is a related IgG endopeptidase that prevents the recognition of IgG bound to *S. pyogenes* by competitively blocking

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IgG from recognition by Fc receptors on host cells. Given that IdeS/Mac have homologs across group A streptococcal strains (8). Streptococcal C5a Protease SepA, is a cell-bound peptidase anchored to the cell wall by sortase A. The streptococcal C5a protease (SCP) is expressed on the surface of all serotypes of *S. pyogenes* and most human isolates of groups B, C, and G streptococci, where it specifically destroys C5a. The enzyme also binds fibronectin and functions as a low level invasion for *S. pyogenes*, group B streptococci and group G streptococci (9).

**Materials & Method**

This study included 200 patients (aged 1-15 years) collected from throat swab who admitted to Al-Hilla Teaching Hospital, during a period extending from January 2018 to December 2018. The specimens were collected from patients with pharyngitis to detect *Streptococcus pyogenes* by bacteriological analysis and vitek 2 system in a proper way to avoid any possible contamination.

**Detection of virulence Genes by PCR technique**

**DNA extraction and purification**: This method was made according to the genomic DNA purification Kit supplemented by the manufacturing company Geneaid, (UK). The suspension containing DNA was stored at-20 C until used as a template for PCR.

**Primer Sequences**: The primer sequences and PCR conditions that are used in the study (10).

**Results and Discussion**

**Isolation of *Streptococcus pyogenes***:

In this study, a total of 200 throat swab were collected from patients suffering from pharyngitis from both sex with age (1-15) years. carried out from ENT unit in Al-Hilla General Teaching Hospital and Al Noor Hospital during the period from January 2018 to December 2018.

The results indicate that the rate of *Streptococcus pyogenes* isolated from a patient with pharyngitis is (15%), others bacterial growth (73%), and no growth (12%). as shown in Table (1).

<table>
<thead>
<tr>
<th>bacterial growth</th>
<th>Numbers of bacterial growth</th>
<th>Percentages %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>30</td>
<td>15%</td>
</tr>
<tr>
<td>Other bacteria</td>
<td>147</td>
<td>73%</td>
</tr>
<tr>
<td>No growth</td>
<td>23</td>
<td>12%</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>100%</td>
</tr>
</tbody>
</table>

The low percentage of *Streptococcus pyogenes* may be due to normal flora that found in the pharynx and other bacteria that cause secondary infection, there compete pathogenic bacteria in nutrient in culture media.

No growth was seen in other 23 samples which indicate the presence of other microorganisms that may be cultured with difficulties such as viruses, fungi and other agents or because of the misuse of antibiotics that cause the disappearance of the bacteria. Antibiotic treatment is recognized as an effective means to reduce transmission of the organism particularly for respiratory and cutaneous infections (11).

**Genetic detection of Virulence factors of *Streptococcus pyogenes* by PCR**

**Molecular detection of (emm gene)**

By using specific two primers for detection of M protein (emm) gene, the result shows that these genes were detected in all isolates bacteria (100%) with molecular length (914 bp) when compared with allelic ladder as shown in figure (1).
Figure (1) agarose gel electrophoresis at 70 volts for 50 min. for \textit{emm} gene in \textit{S. pyogenes}. PCR product visualized under U.V light at 320nm. After staining with ethidium bromide. L: Ladder with 2000 bp. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 were positive for this gene with amplicon size 914 bp.

Khosravi \textit{et al}, (2016) found that the \textit{emm} gene detects in all isolates of GAS isolates isolated from throat samples in children with sore throat, and found that the types of \textit{emm} gene different according to the types of diseases\textsuperscript{(12)}.

\textbf{Molecular detections of pyrogenic exotoxins (\textit{SpeB} ,\textit{SpeC})}

In this study two primers were used to detect the \textit{SpeB} gene and the result show that (100\%) isolates contain the gene with molecular length (952 bp) when compared with allelic ladder as shown in figure (2)

Figure (2) agarose gel electrophoresis at 70 volts for 50 min. for \textit{speB} gene in \textit{S. pyogenes}. PCR product visualized under U.V light at 320nm. After staining with ethidium bromide. L: Ladder with 2000 bp. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 were positive for this gene with amplicon size 952 bp.

Hytonen \textit{et al}.,(2001) found that the \textit{SpeB} gene is carried by all strains of \textit{S.pyogenes}, but the degree of expression varies from strain to strain, and \textit{SpeB} has been considered to produce only in a secreted form. the expression of \textit{SpeB} is controlled by the multiple gene activator \textit{mga}\textsuperscript{(13)}. Also in this study the \textit{SpeC} gene also study by using specific primer at molecular length (405 bp) and the result s show that (75\%) give positive results to this gene when compared with allelic ladder as shown in figure (3)

Figure (3) agarose gel electrophoresis at 70 volts for 50 min. for \textit{speC} gene in \textit{S. pyogenes}. PCR product visualized under U.V light at 320nm. After staining with ethidium bromide. L: Ladder with 2000 bp. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 were positive for this gene with amplicon size 405 bp.
In this result it was found that SpeB is more prevalence than other gene SpeC this may due to that this gene coded by chromosome and SpeB is the key virulence factors in GAS pathogenesis.

Also the distribution in the prevalence these genes may attributed to site of infection or may the isolates contain other genes responsible for exotoxin, which it was found that there are (11) gene responsible for *Streptococcus pyogenes* exotoxin

**Complement membrane attack complex (Mac) gene detected by PCR**

Specific primer was used to amplify the mac gene in all *S.pyogenes* isolates the results show that (30%) give positive, results to this gene with molecular length (389 bp) when compared with an allelic ladder as shown in figure (4)

![Figure (4) agarose gel electrophoresis at 70 volts for 50 min. for mac gene in S. pyogenes. PCR product visualized under U.V light at 320nm. After staining with ethidium bromide. L: Ladder with 2000 bp. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 were positive for this gene with amplicon size 389 bp.](image)

The different the percentage of this gene may be due to the bacterial contain other gene encoded for complement factor degradation other than mac like Endos and C5a peptidase.

**Molecular detection of scpA (streptococcal C5a peptidase) by PCR**

DNA was extracted from (30) *streptococcus pyogenes* isolates, PCR was carried out using these DNA from the amplification of specific primer (scpA) after the gel electrophoresis, the result shows that 17 isolates (56.6%) give positive result to this gene with amplicon size (622) when compared with allelic ladder as shown in figure (5)

![Figure (5) agarose gel electrophoresis at 70 volts for 50 min. for scpA gene in S. pyogenes. PCR product visualized under U.V light at 320nm. After staining with ethidium bromide. L: Ladder with 2000 bp. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 were positive for this gene with amplicon size 622 bp.](image)

scpA decreases the rate of GAS clearance by inhibiting chemotactic recruitment of phagocytic cells to the site of infection, also shown to promote Fn independent GAS invasion of the human epithelial cell (14). The negative results may belong to that the isolates contain another gene responsible for protease gene like (speB and SPYCEP) or gene is non-functional gene.
Conclusion

Humans are the only reservoir for GAS. It is most common among children 5 through 15 years of age, presence of superantigen genes within the S. pyogenes genome suggests that they do play a significant role in S. pyogenes disease, high prevalence of emm, speB, sdaB gene in streptococcus pyogenes isolates.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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References

Iron Deficiency among Patients with Febrile Seizures in Al Ramadi Maternity and Children teaching Hospital, Western Iraq

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Abstract

Background: Febrile seizures are the most predominant neurological disorders in children between 6 months-5 years, at the same time iron deficiency anemia is one of nutritional insult that implicated as risk factor for occurrence of febrile seizures.

Objectives: to estimate the role of iron deficiency as a risk factor for febrile seizures in children aged 6 months -5 years, western Iraq.

Patients and method:- A case control study has been conducted in AL Ramadi maternity and children teaching hospital during January to October of 2016. All children with history of febrile seizures aged 6 months-5years were involved as cases group, others with febrile illness and no seizures were considered as control group. Data from 58 child of each group were collected. The data include age, sex ,temperature, causes of febrile illness, Hb ,hematocrit and s. ferritin levels. The data were subjected to statistical analysis run under IBM SPSS ver. 23.

Results: Means age ± SD were 27.48±14.83 & 23.24±14.37 months in cases and control groups respectively with no significant difference between them. Most of the children (65.52%) of those with febrile seizures were less than 3 years old. Mean temperature of cases group (38.86°C) was found to be significantly greater than that of the control group (38.52°C) at p-value <0.01. Mean Hb level ,PCV%, s. ferritin level were significantly lower in cases group than in control group. Proportion of iron deficiency anemia was significantly higher in febrile seizure group (65.52%) than in control group (31.03%) at p-value< 0.01.

Conclusions: Occurrence of IDA in cases group is found to be more than 2 times of that in the control group. Early detection and proper treatment of IDA can play a prominent role in limitation the prevalence of febrile seizures among children below 5 years.

Key words: febrile seizures, IDA, western Iraq

Introduction

Febrile seizures (FSs) is the most predominant neurological disorders in childhood with a multifactorial inheritance that occur in 2-5% of children in united states,5-10% in India and up to 14% in Guam\(^1\). FSs described as a seizures that come in association with high grade fever of 38 centigrade or more without brain infection or electrolytes disturbances involving age groups ranged from 6months-5 years with peak age of occurrence of 18-24 months\(^4\). \(^5\) 

Iron deficiency anemia (IDA) is another well pronounced easily correctable nutritional problem that can affect the intellectual development and occur predominately in a nearly similar age interval of FSs in young children 6months -24 months\(^6\). \(^7\)
In general, anemia was noted in a proportion 46-66% of children younger than 4 years of age in developing countries and approximately half of them were iron deficient\(^8\).

Indeed, iron is one of the micronutrients that have an important role in neuronal development (neurogenesis), maturation of myelin, energy and neurotransmitters metabolism\(^9,10\) as well as formation of hemoglobin.

Thus abnormal neurotransmitters functions as a sequence of iron deficiency, may in turn contribute to alteration of seizure threshold and initiation of febrile seizures in young children\(^7\).

So if we take into account the same age prevalence of IDA & FSs and the effect of iron deficiency on brain function, as well as the value of Hb in carrying O2 to brain, and since presence of fever can exaggerate the symptoms that result from effect of IDA on brain, so relation between FSs & IDA is probable.

Based on all the above considerations, and since no study was conducted in Al Ramadi province western Iraq about such problem, so our study was conducted to estimate the role of iron deficiency as a risk factor for febrile seizures among children from 6 months-5 years, western Iraq.

**Patients and Methods**

A case control study has been conducted in Al Ramadi maternity and children teaching hospital, western Iraq during the period January to October of 2016. The study approved by the ethics committee of AL Anbar University, Medical College. All children who admitted with history of febrile seizure and their age ranged from 6 months-5 years were involved in the study as a febrile seizure group (FSG). The control group collected randomly from children between 6 months -5 years who they admitted for same hospital during study period with febrile illness & no seizures (non-seizure group, NSG).

Detailed history and physical examination were done for them. Simple febrile seizure defined as single seizure that associated with peak of fever, last less than 15 minutes, once per day and generalized features, complex febrile seizures defined as seizure last more than 15 minutes, frequent per day or had focal features\(^11\). All patients with history of CNS infection and history of delayed developmental milestone were excluded.

Information that collected from studied groups include age, sex, degree of temperature at admission, causes of febrile illness (respiratory tract infection, gastroenteritis, otitis media, tonsillitis, others), nature of seizures, duration and frequency of seizure were all recorded for every patient. Five mls of blood was aspirated to assess Hb, hematocrit (PCV) and s. ferritin values in studied groups (ferritin Accu Bind, ELISA Microwells, USA).

IDA defined as Hb level less than 11gm/dl. PCV less than 30%, s. ferritin less than 12ng/ml\(^6,12\).

The data were statistically analyzed using IBM SPSS v. 23.0, p-value < 0.05 was considered significant.

**Results**

One hundred sixteen children aged 6 months-5 years were included in the study during the period mentioned previously. Patients were classified into two groups each of 58 patients. The cases group involved 34 males and 24 females which indicates a male to female ratio of 1.42:1, on the other hand, the control group consist of 36 males and 22 females with a male to female ratio of 1.64:1.

Regarding FSG, 48 (82.7%) of them had simple type febrile seizures, and the remaining cases had complex type (17.3%). Most of patients with febrile seizures 38 (65.52%) were below age of 3 years. Out of this group 22 (37.93%) their age ranged from 12-24 months. Only four patients (6.9%) were found in the age group 6-12 months (Table 1).

The means age were 27.48±14.83 and 23.24±14.37 in FSG & NSG respectively with no significant difference between them (p =0.120), (Table 2).

The Chi-square test revealed no significant association between gender and groups of the study (Chi-square=0.144, p-value=0.7). Percentages of gender groups were compared to each other in both groups and found to be not significantly different (Table 2). Mean temperature at admission was significantly higher in FSG than NSG, (p =0.120).

Respiratory tract infection was significantly the most predominant cause of febrile illness in FSG 30 (51.7%) as compared to NSG 10 (17.2%), whereas gastroenteritis was significantly lower in FSG 20 (34.5%) as compared to NSG 38 (65.5%) (Table 2).
Means Hb level, PCV% & s. ferritin levels were significantly lower in FSG than in NSG, p values were all lower than 0.05.

The proportion of IDA among FSG was significantly higher than that of NSG, 38 (65.52%) vs. 18 (31.03%), p-value=0.0003.

**Table 1: Distribution of patients according to age, gender and case-control groups.**

<table>
<thead>
<tr>
<th>Age (Months)</th>
<th>FSG (n= 58)</th>
<th>NSG (n=58)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gender</td>
<td>Gender</td>
</tr>
<tr>
<td></td>
<td>Males N(%)</td>
<td>Females N(%)</td>
</tr>
<tr>
<td>6-&lt;12</td>
<td>0(0.0)</td>
<td>4(16.67)</td>
</tr>
<tr>
<td>12-&lt;24</td>
<td>14(41.18)</td>
<td>8(33.33)</td>
</tr>
<tr>
<td>24-&lt;36</td>
<td>4(11.76)</td>
<td>8(33.33)</td>
</tr>
<tr>
<td>36-&lt;48</td>
<td>6(17.65)</td>
<td>4(16.67)</td>
</tr>
<tr>
<td>48-60</td>
<td>10(29.41)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Total</td>
<td>34(100%)</td>
<td>24(100%)</td>
</tr>
</tbody>
</table>

**Table 2: Demographic Characteristic and Hematological Findings Of Febrile Seizure Group and Non-Febrile Seizure Groups**

<table>
<thead>
<tr>
<th></th>
<th>FSG( cases) (N=58)</th>
<th>NSG(Controls) (N=58)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, months(mean)</td>
<td>27.48±14.83</td>
<td>23.24±14.37</td>
<td>0.120</td>
</tr>
<tr>
<td>Gender</td>
<td>Male N(%)</td>
<td>34(59)</td>
<td>36(62)</td>
</tr>
<tr>
<td></td>
<td>Female N(%)</td>
<td>24(41)</td>
<td>22(38)</td>
</tr>
<tr>
<td>Temperature c0(mean ±SD)</td>
<td>38.86 ± 0.544</td>
<td>38.52 ± 0.580</td>
<td>0.001 *</td>
</tr>
<tr>
<td>Causes of febrile illness N(%)</td>
<td>Respiratory tract infection</td>
<td>30(51.7)</td>
<td>10(17.2)</td>
</tr>
<tr>
<td></td>
<td>Gastroenteritis</td>
<td>20(34.5)</td>
<td>38(65.5)</td>
</tr>
<tr>
<td></td>
<td>Otitis media</td>
<td>4(6.9)</td>
<td>4(6.9)</td>
</tr>
<tr>
<td></td>
<td>others</td>
<td>6(10.3)</td>
<td>6(10.3)</td>
</tr>
<tr>
<td>§ Hb g/dl(meanSD)</td>
<td>10.54±0.83</td>
<td>11.16±1.12</td>
<td>0.001*</td>
</tr>
<tr>
<td>§§ Pcv%(meanSD)</td>
<td>30.72±1.58</td>
<td>34.50±3.63</td>
<td>0.000*</td>
</tr>
<tr>
<td>s. ferritin ng/ml (mean)</td>
<td>20.97±11.44</td>
<td>43.59±26.15</td>
<td>0.000*</td>
</tr>
<tr>
<td>IDA N(%)</td>
<td>38(65.52)</td>
<td>18(31.03)</td>
<td>0.0003*</td>
</tr>
</tbody>
</table>

§ Hemoglobin, §§ Packed cell volume, * Significant difference
Discussion

Febrile seizures are the most common neurological disorder that occurs in children less than 5 years old without central nervous system infection or electrolyte disturbances.

In the current study FSs were pronounced predominately in children below age of 3 years (65.52%), mainly among 12-24 months age group which is in agreement with other studies (13,14).

The mean age of onset of FSs was 27.48 months which is nearly similar to others (4,5,15,16).

As described by other researchers (13,17) majority of patients with FSs had simple type of seizure (82.7%) and the remaining had complex type. The association between FSG, NSG and gender groups was statistically insignificant which is comparable with results of other reports (18,19).

Moreover, Like other reports (15,20,21), this study revealed a significant high peak temperature at admission in FSG than NSG. It was generally reported that fever is one the risk factors implicated in occurrence of FSs that may aggravate the worse effect of lack of iron on brain function which may precipitate seizure attack (17,22).

Regarding causes of febrile illness, respiratory tract infection was the predominant cause of febrile illness in FSG which is found to be significantly higher than that of the NSG and that is in agreement with other studies (23-25).

On the other hand gastroenteritis was significantly lower in FSG than NSG which supports the suggestion of its protective effect against FSs as reported by other studies (5,26).

Several previous publications from different countries worldwide clearly demonstrated the existence of association between IDA and FSs and they considered IDA as an important risk factor for occurrence of FSs (9,27,28).

This study carried out in the Western region of Iraq in order to cast light on some of the important concepts associated with main issue of the study. Accordingly, this study showed that there are statistically significant reduction in means Hb level ,PCV %, s. ferritin level of (FSG) compared to (NSG), in addition the proportion of IDA in FSG was more twice that in the NSG (table 2).

This results is in agreement with Daoud A S etal’s report, from Jordan, 2002 (29) who study the association between IDA & s. ferritin ,they thought that lack of iron could be possible cause of FSs. as they found a significant low mean s. ferritin levels and higher percentage of cases with low serum ferritin 30ug/dl among FSG than controls ,29±21mcg/L vs. 53.3±37.9mcg/L , ( p 0.000).

On other hand, Kumari et al, 2012 (30) used univariate and multivariate analysis for the data collected from the same purposes of this study, they observed a significant higher proportion of iron deficiency in FSG than controls, 63.6% vs.24.7% , p=0.001 which is complies with the results of the current study. The same conclusion reported by Momen A et al., 2010 from Iran (18) whom they confirm the existence of positive relation between febrile seizure and IDA.

Habibian N ,et al., 2014(31) conducted a meta-analysis study and observed that IDA was moderately increased the chance of FSs in children especially in the regions of low or moderate percentage of IDA.

Moreover, similar significant association between IDA &FSs was pronounced by El-Shafie et al., (32), 2017 whom conducted a prospective case control study of 60 cases aged 6 months -5 years from Egyptian children, they found that 21 (52.5%) of cases had IDA compared to 4 (20%) of controls p 0.05.

However, other studies had proved otherwise and denied any association between IDA and FSs and thus did not agree with results of this study. Amirsalari etal(33) Omen etal (34) and Abaskhanian A etal(35) deduced a lack relationship between IDA and FSs.

Furthermore, Derakhshanfar H, etal (5) suggested that IDA was less common in febrile seizure patients than healthy children. Whereas Talebian A ,etal (36) in a study involved 120 children aged less than 5 years, reported that IDA was more common in controls than FSG and concluded that IDA was not a risk factor but rather a protective factor against FSs.

Bidabadi E and Mashouf M,(15) in a case control study of 200 cases with febrile seizures and 200 controls aged 6 months-5 years, found that IDA was less frequent in patients with FSs than controls with no statistical difference between the two groups, but the protective effect of iron deficiency against FSs was not proved (odd ratio 1.175).
Indeed, these variation in results of these reports could be related to the variations in studies designs, different samples sizes and age groups involved, different measures used for diagnosis of IDA, nutritional status and prevalence of IDA among children involved in these studies from different regions. Unfortunately, no previous study was carried out in al Ramadi province, Western Iraq, regarding prevalence of IDA among children aged less than 5 years. Based on the results of this study, it can be concluded that IDA is about two times more predominant in FSs patients than in other patients with no FSs, and Iron deficiency is well recognized as a risk factor in children aged less than 5 year. In this context, early detection with proper correction of IDA will be effective in reducing rates of FSs among those children. Large sized prospective study is necessary to assess prevalence of IDA in children less than 5 years as well as to help evaluating nutritional problems that may contribute positively to the IDA.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References


Antibiotic Susceptibility of Bacteria Isolated From Under Nails

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Abstract

This study was designed for isolation and diagnosis of bacteria from under long dinger nails of a wide population of student (kindergarten, primary schools, elementary schools and university students) and study the antibiotic sensitivity for isolated bacteria.

From 100 sample the bacterial isolate were Staphylococcus sp.(56 isolate), Bacillus sp.(1 isolate), Streptococcus sp. (1 isolate), Escherichia coli (40 isolate), Salmonella (2 isolate), Enterobacter (10 isolate), Klebsiella (10 isolate), Serratia (5 isolate) and Pseudomonad (6 isolate).

The most effected antibiotic on all types of bacteria isolated from under the long nail was Gatifloxacin and the lowest effect was Cefazolin antibiotic.

The concept of the study was isolating and determining bacteria, found under the long fingernails and studying their antibiotic sensitivity.

Keywords: Under Nails; Antibiotic; Bacteria Isolated; Health.

Introduction

The skin on the human body is in permanent contact with the environmental microorganisms, these contaminant microorganisms can easily be isolated in laboratories. These microbes can induce a range of diseases in the community or hospital, including urinary tract, respiratory tract, injuries and burns, bacteremia, neonatal meningococcalpitalis, empyema and osteomyelitis. The hand acts as a significant transmission platform for different microbes, including the enteric species [1].

The most in touch human body components with the outside world are the hands. People are using their hands every day for a wide range of different activities. Contacting distinct microbes and transferring them to other objects and perhaps even individuals is highly simple. Surprisingly, the larger number of bacteria discovered on human hands are under fingernails [2]. In many health-related problems, fingernails are progressively seen as a significant concern due to the ability to harbor many kinds of microorganisms [3].

The finger nail is a significant structure consisting of the protein, keratin, laminated layers. Nails have two key roles, in spite their tiny number. They function as a protective lamina and by acting as a counter-force to improve the feeling of the fingertip. Every nail comprises several components including: nail root, nail bed, nail plate, perionychium and hyponychium [4].

Even microbes may still occur under fingernails when hands are washed. Higher microorganism populations (2 to 3 log CFU / fingernail) happen commonly under the nails and are often more hard to remove than at other hand places (CDC, 2002). Length and texture of the fingernails also influence the effectiveness of microbial removal from below the nails. Long and polished nails usually contain more microbes after cleaning hands than brief and unpolished nails [5].

Therefore, using artificial fingernails can be a factor that influences the effectiveness of hand washing as artificial nails are generally longer than natural nails. Several studies have shown that greater populations of microbial are retrieved from artificial nails than natural nails [3]. Effective hand washing techniques are crucial in
stopping disease transmission via finger nails to remove microbes from artificial or natural [6].

The higher population of pathogenic microorganisms found under long nails were Escherichia coli, Shigella, Salmonella, Enterobacter, Klebsiella, Serratia, Proteus, Bacillus and pseudomonas [6, 7].

Materials and Method

Collection of samples

A total of 100 samples were collected from Kindergarten students, primary, secondary and university students. This study was conducted between October 2017 and May 2018 in Al-Hillah city. The samples were gathering by sterile tooth picking, the tooth picking was scrub throughout the surface of the under-nails and moved to the brain heart infusion broth to guarantee that bacteria remained alive, after the broth was incubated at 37°c for 18 h.

Methods of isolation and diagnosis

Culturing Methods

In the research, the nutrient agar medium was used for bacterial culturing. Detailed data was gathered under the samples of the nails (lengthy nails) depending on era and sex. The crops were incubated at 37 ° C and bacterial development was inspected at 24 hours. To achieve pure culture, separate colonies were sub-cultured into nutrient agar. Shape and color of colonies (morphological characteristics), gram stain tests and biochemical tests were used to identify bacteria.

Identification of Bacteria

Bacteria were diagnosed by using several selective media like, mannitol salt agar, maCconky agar, eosin methylene blue, SS agar and blood agar also bacteria were gram stained.

Antibiotic Susceptibility Test

Several antibiotic dicks were used (Aztreonam ATM(30mcg), Cefazolin CZ(30mcg), Cefotaxime CTX(30mcg), Clindamycin DA(2mcg), colistin CT(10mcg), Gatifloxacin GAT (5mcg), Nitrofuranton F(300mcg) and Trimethoprim/ Sulphamethoxazol SXT(25mcg)) to test the sensitivity of bacteria. The test was done by using Muller Hinton media.

Result and Discussion

Microorganisms existence under the nails has become the most widely health issue. A total of 100 samples from under nails by tooth picks were gathered from under the nails, 100 students were gathered in the left and right hands.

All students (100%) found that they were harboring bacteria under their nails. Bacterial pathogens that were isolated from the students ' lower nails were found in (table 1).

Table (1): Types and Number of bacteria isolated from samples under nails

<table>
<thead>
<tr>
<th>No.</th>
<th>Gram – positive</th>
<th>Gram – negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Staphylococcus sp.</td>
<td>56</td>
</tr>
<tr>
<td>2.</td>
<td>Bacillus sp.</td>
<td>1</td>
</tr>
<tr>
<td>3.</td>
<td>Streptococcus sp.</td>
<td>1</td>
</tr>
<tr>
<td>4.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>Total</td>
<td>58</td>
</tr>
</tbody>
</table>

Rayan and Flournoy Clarify the presence of large bacterial growth under fingernails over 1 mm in length, showing that volunteers with short finger nails (cut correctly) had 64% bacterial contamination (bacterial count) and volunteers with lengthy finger nails had more (67%) bacterial count presence on their hands [8].

Lin indicated that more microorganisms tend to harbor lengthy fingernails than brief nails. Visibly smooth nails were only noted by the appearance of students ‘ finger nails, showing 62% bacterial contamination on their hands. Ray noted there was a reduction in the bacterial isolates after washing hands with soap [9, 10].

Tambekar also found the largest bacterial contamination (70%) in the hands of Kindergarten volunteers followed by 67% in the hands of primary volunteers, 66% in the hands of secondary pupils, 64% in the hands of PG volunteers and at least (57%) in the hands of undergraduate volunteers [11].
The microorganisms that isolated and diagnosed from fingernail tested for antibiotic sensitivity, eight antibiotic were used as mentioned in material and methods, (antibiotic resistance pattern were shown in table (2)).

### Table (2) antibiotic resistance pattern of isolated bacterial species from finger nail (compared with NCCLS guidelines)

<table>
<thead>
<tr>
<th>No.</th>
<th>Bacterial strains</th>
<th>Clear zone diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CZ</td>
</tr>
<tr>
<td>1.</td>
<td>Pseudomonad</td>
<td>R</td>
</tr>
<tr>
<td>2.</td>
<td>Klebsiella</td>
<td>R</td>
</tr>
<tr>
<td>3.</td>
<td>Streptococcus sp.</td>
<td>R</td>
</tr>
<tr>
<td>4.</td>
<td>Escherichia coli</td>
<td>R</td>
</tr>
<tr>
<td>5.</td>
<td>Salmonella</td>
<td>R</td>
</tr>
<tr>
<td>6.</td>
<td>Staphylococcus sp.</td>
<td>R</td>
</tr>
</tbody>
</table>

**Diagram (1) Clear zone diameter by (mm) for different strain and antibiotic types**

As it is clear from the diagram above Gatifloxacin was the most effected antibiotic on the bacteria isolated from under nails and the lowest effect was belong to Cefazolin.

Kibret M. Says *E. Coli* isolates show high erythromycin, amoxicillin and tetracycline resistance levels. For experimental treatment of *E. coli*, nitrofurantoin, norflaxacin, gentamicin and ciprofloxacin are regarded suitable *E. coli* in the field of research. It is recommended to monitor regularly antimicrobial susceptibility [12] while the result of this study, *E. coli* show resistant to all antibiotics that had been studied.

The results of *Salmonella* sensitivity test show high level of resistance to cefazolin, nitrofurantion, clindamycin, cefotaxime and trimethoprim/sulphamethoxazole and had mild sensitivity to colistin, gatifloxacin and aztreonam; Mijovic etal noticed that there were increase in the susceptibility rate to many antibiotic between their two surveys [13].

The sensitivity was tested in vitro and on the basis of laboratory results, antibiotics were given to patients. Over the past 21 years, *Klebsiella* has shown a substantial shift in sensitivity pattern. These organisms' sensitivity to different antibiotics tested has decreased over time. The organism carries out most of the in vitro antibiotics it is subjected to. Srinivasan and his coworkers explained their efforts should be focused on reducing the use of antibiotics or a correct antibiotic policy that controls the meaningless and excessive use of antibiotics [14]. their fears about gaining bacteria multi-resistance drugs traits had been observed in this research as the *Klebsiella* isolate was resistant to all eight antibiotics used.

*Pseudomonas* antibiotic susceptibility test appear that *Pseudomonas* strains were resistance to almost all antibiotics and sensitive only to gatifloxacin, as clear from this study *Pseudomonas* resistant to colistin while Sader and his coworkers maintained that *Pseudomonas* show sensitivity >90% to colistin in the time between 2012-2015 [15].

*Streptococcus* isolate that obtained from this study was resistant to Aztreonam, Cefazolin, Clindamycin, colistin, Nitrofurantion and Trimethoprim/Sulphamethoxazole, while it was sensitive to Cefotaxime, Gatifloxacin, The highest resistance *Streptococcus* showed to erythromycin, clindamycin and trimethoprim-sulfamethoxazole and these should be avoided in the treatment [16].

*Staphylococcus* colonies showed resistance to most antibiotic that had been used in the study as listed in table (2), *Staphylococcus* spp. Also show sensitivity to three types of antibiotics (Nitrofurantion, Gatifloxacin and Clindamycin), many researchers reported that *Staphylococcus* which isolated from finger nail have multiple antibiotic resistant [6, 17, 18].
**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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### References


Removal of Tetracycline from Aqueous Solutions using Pomegranate Peels Residues Accessing to ZRL

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Abstract

Antibiotics are harmful pharmaceuticals to ecosystems in general and aquatic systems in particular. Therefore, its remediation from water bodies is a topic of great importance for water treatment and purification workers. This research investigates the removal of one of the most famous types of antibiotics, which was tetracycline from simulated synthetic aqueous solutions by adsorption technique using non-toxic, low cost and available agricultural waste which was pomegranate peels. The adsorption experiments were performed in adsorption laboratory unit of batch mode at different operating conditions and laboratory temperature. The operating parameters studied included pH of solution, dose of adsorbent media, treatment time, agitation speed and initial concentration of tetracycline. The results showed the ability of pomegranate peels to extract tetracycline from aqueous solutions with high efficiency of 81.55%. The results also showed that the percentage of antibiotic adsorption from aqueous solutions was inversely correlated with increasing the initial concentration and acidic function of the tetracycline solution while it was directly proportional to the amount of pomegranate peels, agitation speed and treatment time. In this style, one of the most important types of antibiotics that contaminated water was disposed of by a cheap material and using a simple, economical and environmentally friendly method accessing to the principle of zero residue level (ZRL).

Keywords: Antibiotic, Tetracycline, Adsorption, Pomegranate peels, ZRL

Introduction

Antibiotics are defined as chemical compounds used in the treatment, prevention and diagnosis of diseases, thus preserving the physical and mental health of both humans and animals (1). Today, these products pose a threat to humans and the environment as they are increasingly present in the aquatic environment, even at low concentrations of up to parts per million (ppm) as a result of increased production due to overconsumption without any treatment before disposing (2). It enters wastewater with urine and excrement as well as industrial waste from pharmaceutical plants also; their environmental impact is increasing when there is a mixture of these substances with metabolites (3). Recent studies have confirmed the inclusion of these formulations as contaminants, as they pose a threat to groundwater and surface water, thus causing adverse effects on wildlife and aquatic life (4). These compounds include, for example, Nonsteroidal anti-inflammatory drugs (NSAIDs), antipyretics, antidepressants, diuretics, antibiotics, and anti-ulcers, whose metabolism produces new compounds that are also polluting the environment. Antibiotics come out with urine in low concentrations, and their metabolites come out with either with urine or excrement (5). Conventional methods of wastewater treatment are ineffective in eliminating the contaminated effect of pharmaceutical compounds due to the resistance of some types or metabolic products to biodegradation (6). Apart from traditional treatment methods, adsorption technology has recently received widespread attention as one of the candidate methods for solving the problem of water contaminated by antibiotics for its ease, efficiency and low cost (7). Studies have shown that the use of adsorption technique by activated carbon is very effective in the disposal of many organic pollutants such as dyes, pesticides and aromatic compounds generally (8). However, there are two problems facing this promising technique: the first problem is the high production cost of activated carbon and needed for continuous regeneration process, in addition to the part loss of this material during each regeneration process, while the second problem relates to the difficulty of sediments
disposal from the surface of activated carbon or other adsorbents (9). This led the researchers to seek for other sources to be used as adsorbents or raw materials in the preparation of activated carbon from them and also so that the amount of remaining materials are small (10). In the last years, the concept of zero residue level (ZRL) has been applied to remedy all the problems associated with the use of adsorption technology. This concept uses the non-valuable waste as adsorption media and then utilizes from the residue adsorption process so that the amount of residual waste is close to zero (11). The present paper aims to use the adsorption technique and the application of the principle of ZRL in the treatment of water contaminated with tetracycline, one of the common types of antibiotics through the use of pomegranate peels as a low-cost adsorbent in an economical, beneficial, low-cost and environmentally friendly method.

2. Experimental Work

Materials

2.1.1 Pomegranate peels (sorption media): The mature pomegranate peels used in this investigation were obtained from juice shops and cafes in Baghdad city as well as from domestic usage. After collection, pomegranate peels (in its originally size) were washed with excess tap water for several times before being washed with distilled water at normal temperature to get rid of any kind of impurities and dust that might be stuck to them. The washed peels were dried naturally by exposing them to the open air and sunlight for uninterrupted 48 hours and then placed in a metal bowl, immersed in fresh water and heated until boiled to remove the color and dye (tanner) from them. Finally kept in dark brown glass bottles and placed in the fridge until used.

3.1.2 Stock solutions: Real water contaminated with tetracycline (a studied antibiotic) contains many compounds and other elements that may be difficult to detect exactly or identify accurately in that type of water. Therefore, the adsorption experiments were carried out using simulated synthetic aqueous solutions (SSAS) containing tetracycline in various concentrations in order to evade nip up with any other kind of pollutants. For this purpose, an aqueous solution, called stock solution, of 1000 ppm of tetracycline, was prepared. In a volume of one liter of distilled water, 1 g of tetracycline powder packaged in capsules was dissolved to obtain the stock solution. All different concentrations SSAS of tetracycline antibiotic used in this research were prepared from dilution of the stock solution with distilled water to the desired concentration. Tetracycline concentrations were determined spectrometrically at a wavelength of $\lambda=529$ nm using a spectrophotometer according to the method described by (12). Figure 1 shows the spectrophotometer calibration curve intended for tetracycline concentrations.

![Figure 1 Spectrophotometer calibration curve of tetracycline @ wavelength of $\lambda=529$ nm](image)

3.2 Adsorption unit: In order to identify the behavior of pomegranate peels as an adsorption media and to determine the best operational conditions that achieve the maximum tetracycline removal percentage, a concatenation of functional experiments were conducted in a laboratory adsorption unit of batch mode. In each experiment 100 ml of tetracycline solution was prepared and laboratory experiments were carried out at different operational conditions of initial concentration of tetracycline, pH of SSAS, amount of adsorbent, agitation speed and contact time. Their ranges were from (1-50) ppm and (1-8) (0.25-2.5) g of pomegranate peels, (100-400) rpm and (10-150) min. respectively and at laboratory temperature (28±2) °C. Each experiment was triplicate to increase accuracy and to reduce the experimental error. To calculate the residual tetracycline concentration at the end of the experiment, the aqueous solution was filtered using vacuum filtration to separate any residue of pomegranate peels may be present in the treated solution. A sample of the filtered aqueous solution was drawn, tested by the spectrophotometer and the concentration of the antibiotic removed was detected. The efficiency of tetracycline removal from the SSAS was determined by calculating the percentage of removal that can be found from the following mathematical relationship:
Where: refers to tetracycline percentage removal, and refer to initial and final concentration of tetracycline (ppm) respectively.

Results and Discussions

As illustrated above, the study of adsorption technology as a suggested remediation method for SSAS contaminated with tetracycline antibiotic was conducted in a batch adsorption unit. The removal process was examined at different operating conditions and using pomegranate peels as a cheap and available adsorbent. This section discusses the effect of the operational conditions used on the tetracycline removal efficiency and determination of the optimum conditions for the maximum treatment efficiency of contaminated SSAS.

3.1 Effect of Initial Concentration of Tetracycline:
The results obtained from the experiments of changes the initial concentration to remove tetracycline using pomegranate peels as an adsorption media from SSAS showed that the percentage removal was increased by decreasing the value of the initial concentration and vice versa. The results also showed that the maximum percentage removal was 81.46% at the lowest initial concentration of 1 ppm while the percentage removal at the highest studied concentration which was 50 ppm was 23.19% as explained in Figure 2. Adsorption technology is great dependent on surface area and it is a constant property of adsorption media, representing the available sites on the surface of the adsorbent material at which adsorption process occurs. The number of these active sites is limited in the adsorbent and has a constant adsorption capacity for a specified number of contaminated matter particles. In the case of low concentrations of tetracycline, the pomegranate peels was able to adsorb more molecules than if the antibiotic concentration is higher; i.e., the number of non-adsorbed tetracycline molecules that will remain free in the solution will be lower at low concentrations at constant volume and therefore the efficiency of pomegranate peels as a medium of adsorption to remove tetracycline will be reduced by increasing the initial concentration of the contaminant in aqueous solutions.

\[
%R = \frac{C_i - C_f}{C_i} \times 100
\]

Figure 2 Effect of initial concentration on the percentage removal of tetracycline antibiotic

3.2 Effect of pH: Figure 3 shows the behavior of tetracycline adsorption process by pomegranate peels when the pH of SSAS is changed. It’s obvious from above Figure that the relationship between the percentage removal and the pH of solution is inversely and the maximum percentage removal was obtained at the lowest pH value. The pH has a clear effect on the adsorption process, as it affects the charge and ionization degree of the active sites at the surface of adsorbent media. This may be due to the dependence of tetracycline ionization on the value of the pH. On the other hand, increasing the pH leads to an increase in the concentration of negative hydroxide ions (OH⁻) in the solution, which generates repulsive forces between them and tetracycline molecules and thus competes for the active sites in the adsorbent, which is already limited. Therefore, the high percentage removal means that there are little competition and repulsion forces between tetracycline and hydroxide. In addition, the surface of the pomegranate peels will be ionized with positive hydrogen ions. This makes the adsorption process easier than if the number of hydroxide ions increases. This happens when the acidic function of the solution is raised.

Figure 3 Effect of pH on the percentage removal of tetracycline antibiotic
3.3 Effect of Adsorbent Amount: The experimental results related to the study of the pomegranate peels effect as an adsorbent on the percentage of adsorption showed that the latter is increasing by increasing the amount of adsorbent by keeping the other operational parameters at optimum values. The maximum removal was recorded at the largest amount of adsorbent, which is 2.5 g, as shown in Figure 4. The direct correlation between the percentage of tetracycline removal and the amount of pomegranate peels is due to the fact that pomegranate peels have a specific surface area per unit weight. Increasing the amount of adsorbent will increase the surface area of the adsorption medium, which means more active sites, which in turn will provide a greater chance of adsorption of more molecules of tetracycline if the amount of pomegranate peels is less.

![Figure 4 Effect of adsorbent media amount on the percentage removal of tetracycline antibiotic](image)

3.4 Effect of Agitation Speed: The correlation between the percentage removal of tetracycline and different values of agitation speed is shown by Figure 5. Increasing the agitation speed will increase tetracycline removal from aqueous solution using pomegranate peels as adsorption medium and keep the rest of the operational variables at optimal values. This results may be attributed for Increasing the agitation speed will reduce the thickness of the layer surrounding the adsorbent molecules, removing the surrounding obstacles and increasing the chance that tetracycline will bind to the active sites on the surface of the pomegranate peels, thus increasing the tetracycline removal from the aqueous solutions. This explanation is true up to the speed of 300 rpm, after this value the percentage removal remains constant and does never change whatever increasing the agitation speed. This may be due to the material is saturated with adsorbed molecules at optimum speed and that any increase in speed will not change the removal efficiency.

![Figure 5 Effect of agitation speed on the percentage removal of tetracycline](image)

3.5 Effect of Contact Time: The increase in contact time leads to a corresponding increase in the percentage of tetracycline removal from aqueous solutions as shown in Figure 6, with keeping the rest of the operational variables at optimal values. Increasing the process time will increase the time when tetracycline molecules come into contact with pomegranate peels, which in turn will increase the chance of tetracycline binding to active sites on the surface of the adsorbent and thus increase the rate of antibiotic removal from aqueous solutions. If the time is less, vice versa, the adsorbent molecules do not find the time required to complete the adsorption process of the molecules of the contaminant and thus remain free in the solution, which leads to a decrease in the percentage of adsorption.

![Figure 6 Effect of contact time on the percentage removal of tetracycline](image)

4. Conclusions: From the results of the present study, the following conclusions can be drawn:

- Adsorption technique showed high efficiency in the treatment of aqueous solutions contaminated with antibiotics in general and tetracycline in particular.
- The maximum percentage removal was 81.46% at 1 ppm of the initial concentration of tetracycline, pH of 1, pomegranate peels amounts of 2.5 g, agitation
c. Adsorption of tetracycline from aqueous solutions using pomegranate peels was directly proportional with the amount of adsorbent (pomegranate peels), agitation speed and contact time to a certain level and then constant. While the percentage removal was inversely proportional to the initial concentration of tetracycline and the pH of the aqueous solution.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Complications of Laparoscopic Cholecystectomy in a Sample of Patients Admitted to Al-Ramadi Teaching Hospital, Anbar-Iraq

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Abstract

Laparoscopic cholecystectomy is currently thought as a surgical procedure that can be done with less risk of complications. However, intraoperative and postoperative complications were existing in 56 (15.01%) patients. Open surgery is the case when laparoscopic cholecystectomy fails to be proceeded with due to many causes such as difficult Calot triangle dissection and empyema of gallbladder.

The high occurrence of cholecystitis in the group of patients aged 40-60 (56%) with 151 female and 59 males initiating the floor to many arguments that may help give a good understanding for such a problem.

Iatrogenic perforation of gallbladder was the most common cause of intraoperative complications. Wound infection was the most cause of postoperative complications. Difficult Calot triangle dissection was the most common cause to convert to open surgery.

Keywords: Hospital; Laparoscopic cholecystectomy ; Infection.

Introduction

In the treatment of benign gallbladder disease, laparoscopic cholecystectomy became the first choice that may be taken instead of open surgery. On the other hand, surgeons believed that this procedure involved high risk of injury compared to open cholecystectomy. In this context, experienced surgeons are needed to proceed with such new technique of treatment.

It has been estimated that in USA about one million patients annually diagnosed with gallbladder disease in which about 75% of them underwent laparoscopic cholecystectomy.

Complications of this treatment procedure may vary according to the health status of patients, experience of surgeons, the post-operative care, as well as many other things that may affect negatively the success of the procedure. In this study a light will be casted on most of the common complications as recorded from patients underwent laparoscopic cholecystectomy.

In this technique a gallbladder is removed by a keyhole-sized incision usually using two 10mm ports and two 5mm ports in which this procedure believed to result with less postoperative complications.

This study aims to evaluate the intraoperative, postoperative complications and rate of conversion treatment in patients with cholecystitis.

Patients and Method

During the period Jan. 2012 to the end of Dec. 2013, 373 patients (102 male and 271 female) with history of cholecystitis admitted to AL-Ramadi General Teaching Hospital, Anbar province, Iraq, were considered in this study.

Data collected from each patient included age, gender, clinical signs and symptoms as well as relevant examinations. Medical investigations such as WBC counts, ultrasound findings, postoperative histopathological findings of gallbladder were recorded.

The collected data were classified in tables according to the purpose of presentation and descriptive and inferential statistics were made whenever needed in this paper.
Results

Table 1 shows the distribution of patients according to age groups with reference to their gender groups. Age ranged between 18-70 years with mean age of 49.28 years. Most of the patients were female and accounted for 72.65%. Patients aged 40-60 constitute the highest percentage among all other.

Table 1. Distribution of patients according to gender and age groups.

<table>
<thead>
<tr>
<th>Age in years</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 20</td>
<td>0</td>
<td>6</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>20-40</td>
<td>31</td>
<td>82</td>
<td>113</td>
<td>30</td>
</tr>
<tr>
<td>40-60</td>
<td>59</td>
<td>151</td>
<td>210</td>
<td>56</td>
</tr>
<tr>
<td>More than 60</td>
<td>12</td>
<td>32</td>
<td>44</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>102</td>
<td>271</td>
<td>373</td>
<td>100</td>
</tr>
</tbody>
</table>

Out of the total patients involved in this study, 56 (15.01%) showed different complications. Table 2 shows the distribution of those patients according to gender and age groups. Males were accounted for relatively higher percentage 55.4% compared to 44.6% of females. About 88% of those patients were in the ages of 40 years or more.

Table 2. Distribution of patients with complications according to age and gender groups.

<table>
<thead>
<tr>
<th>Age in years</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20-40</td>
<td>4</td>
<td>3</td>
<td>7</td>
<td>12.5</td>
</tr>
<tr>
<td>40-60</td>
<td>19</td>
<td>9</td>
<td>28</td>
<td>50.0</td>
</tr>
<tr>
<td>More than 60</td>
<td>8</td>
<td>13</td>
<td>21</td>
<td>37.5</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>25</td>
<td>56</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Recorded complications are classified into two main categories, intraoperative and postoperative as presented in table 3. Out of the total patients of this study, 22 (5.9%) revealed intraoperative complications whereas 34 (9.12%) showed postoperative complications. Out of the intraoperative complications, 7 cases (22.58%) found to have vascular injuries. Iatrogenic perforation of GB was accounted for the highest percentage (41.94%) among all other types of intraoperative complications.

With regard to postoperative complications, wound infection was found the most common complication which accounted for 30.43% followed by bleeding from abdominal cavity with 21.74%.

Table 3. Intraoperative and postoperative complications classified by gender groups (n=373).

<table>
<thead>
<tr>
<th>Complications</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraoperative:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vascular injury</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bleeding from bed of GB</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>1.34</td>
</tr>
<tr>
<td>bleeding from cystic artery</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0.27</td>
</tr>
<tr>
<td>bleeding from port site</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0.27</td>
</tr>
</tbody>
</table>
Causes of conversion to open surgery are not identical, rather they vary from patient to another. In this study, only 20 (5.36%) patients were transferred to open surgery under different reasons as presented in table 4. The most common cause was difficult calot triangle dissection which accounted for 35% followed empyema of gall bladder with 25%.

Table 4. Causes of conversion to open surgery (n=373).

<table>
<thead>
<tr>
<th>Causes of conversion to open surgery</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difficult calot triangle dissection</td>
<td>4</td>
<td>3</td>
<td>7</td>
<td>1.88</td>
</tr>
<tr>
<td>Empyema of gall bladder</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>1.34</td>
</tr>
<tr>
<td>Bleeding from vascular supply</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>0.8</td>
</tr>
<tr>
<td>Mirrizzi syndrome</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0.54</td>
</tr>
<tr>
<td>Injury to CBD</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0.54</td>
</tr>
<tr>
<td>Injury to common hepatic duct</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0.54</td>
</tr>
<tr>
<td>Cholecystoduodenal fistula</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0.54</td>
</tr>
<tr>
<td>Colonic injuries</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0.54</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>9</td>
<td>20</td>
<td>5.36</td>
</tr>
</tbody>
</table>
It may be worthwhile referring the cases that presented with different complications to the medical procedure on which the decision of laparoscopic cholecystectomy was decided. In this context, table 5 showed the cases according to the type of complication.

With regard to WBC counts, cases with more than 10000/mm³ were more than others for all categories of complications. Acute cholecystitis was accounted for greater number of patients at all categories of complications for both ultrasound and histopathological findings.

Table 5. Cases with different complications classified with respect to medical procedures (n=373).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Complications</th>
<th>Intraoperative</th>
<th>Postoperative</th>
<th>Conversion to open surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC count</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>More than 10000/mm³</td>
<td>15 (4.02%)</td>
<td>22 (5.9%)</td>
<td>12 (3.22%)</td>
<td></td>
</tr>
<tr>
<td>Less than 10000/mm³</td>
<td>7 (1.9%)</td>
<td>12 (3.22%)</td>
<td>8 (2.14%)</td>
<td></td>
</tr>
<tr>
<td>Ultrasound findings</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic cholecystitis</td>
<td>9 (2.41%)</td>
<td>13 (3.5%)</td>
<td>6 (1.61%)</td>
<td></td>
</tr>
<tr>
<td>Acute cholecystitis empyema</td>
<td>13 (3.5%)</td>
<td>21 (5.63%)</td>
<td>14 (3.75%)</td>
<td></td>
</tr>
<tr>
<td>Histopathological findings</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic cholecystitis</td>
<td>10 (2.68%)</td>
<td>15 (4.02%)</td>
<td>9 (2.41%)</td>
<td></td>
</tr>
<tr>
<td>Acute cholecystitis</td>
<td>12 (3.22%)</td>
<td>19 (5.09%)</td>
<td>11 (2.95%)</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Since its first introduction in 1985, laparoscopic cholecystectomy is rapidly becoming very common technique. This technique enables surgeons to avoid complications of open surgery as well as the high cost of staying at hospitals waiting for recovery.

Most of the patients considered in this study were in the range of 18-70 years with mean age of 49.28 years. This mean age is less than that obtained by Chay CH, et al., 2006 (56.9 years) which also showed a wider age range (23-89 years)³. However, mean age of patients considered in this study is found to be less than that obtained by Al-Salamah SM, 2005⁶. Such a discrepancy can be either attributed to the sampling technique used to select the right patient, or to a certain situation that maybe the sample’ population concerned about. In Iraqi communities, people after 20 years of age become responsible about their families affairs and that they have to do more efforts in order to put their families in a good economic stands. As a result, they become gradually confronted with different health problems due to the food which is not healthy prepared or even do not suit their real needs.

Vascular injury is one of the intraoperative complications, the percentage of this type of complication in this study was found to be 1.88 which is much lower than that (9.97) obtained by Rooh-ul-Muqim, et al,2008⁹.

Iatrogenic perforation of gallbladder was found to have the highest percentage (3.49) among all other causes on intraoperative complications. This percentage is doubtless significantly lower (25.5) than that obtained by Zubair M, et al, 2010¹⁰. Iatrogenic perforation of gallbladder may be associated with the adhesion in right upper quadrant of gallbladder, or with other variables such as gender which is not the aim of this study.

Spillage of gall stones was found in 1.88% of patients in this study. This percentage was also much lower than that obtained by Zubair M, et al, 2010 which was 11.5. However, the percentage 1.88 was almost similar to that (2.02) obtained by Miodrag Radunovic, et al, 2016¹¹.

It is believed that the association of iatrogenic perforation of gallbladder and spillage of gall stones may
lead to abdominal infections which in turn can result in a number of abdominal problems.

With regard to the postoperative complications, the most type was found to be wound infection which was found in 14 (3.75%) patients of this study. Such problem may be caused by poor hygiene or to polluted environment where the patient(s) moved to after surgery. The second cause of postoperative complication was bleeding from abdominal cavity which occur in 10 (2.68%) patients in this study. The third type of postoperative complications in this study was the bile leak which found in 7 (1.88%) patients which is less occur when compared to 3.98% of patients in the study conducted by Rooh-ul-Muqim, et al.,2008.

Other types of postoperative types as found by this study seems very rare and the deep vein thrombosis do not appear to be mentioned in literature reviewed during the research.

Causes of conversion to open surgery are also vary according to the patient situation as well as potential complications during key-hole-surgery. However, in this study the main causes were difficult calot triangle dissection which was seen in 1.88% of the patients and empyema of gallbladder which seen in 1.34% of the patients. In general, 5.36% of the patients of this study were converted to open surgery and when compared to 3.13% that found by Rooh-ul-Muqim, et al.,2008, one may easily conclude that patients of this study were actually confronted by many problems that make their health stands in a serious situation.

Number of patients with intraoperative and postoperative complications as well as of those converted to open surgery, were cross-classified with respect to medical signs and findings. Patients with WBC counts more than 10000/mm³ are remarkable more than those with WBC counts lower than 10000/mm³. Acute cholecystitis was also found in a number of cases that essentially exceeds those with chronic cholecystitis. This is true for both ultrasound and histopathological findings.

**Conclusion**

Laparoscopic cholecystectomy is indeed well prevailed in surgical practice. It is not always the only good procedure to follow but a procedure with less pain and potential complications. Nevertheless, over the wide use of this procedure, different types of complications were recorded. Actually, complications may appear due to variety of factors including the health status of the patients.

In this study most of the admitted cases to undergo laparoscopic cholecystectomy were over 29 years of age and 68% of them were above 40 years old with male to female ration of 0.38:1.

Complications found in 56 of the patients, of them 31 males and 25 females.

Iatrogenic perforation of gallbladder, spillage of gall stones and vascular injury were the most common causes of intraoperative complications. On the other hand, wound infection, bleeding from abdominal cavity, bile leak, pneumonia and loss of gall stones in abdominal cavity were the most common causes of postoperative complications.

Difficult calot triangle dissection and empyema of gallbladder were the most common causes of conversion to open surgery.

Most of those converted to open surgery have WBC counts more than 10000/mm³ and acute cholecystitis in both ultrasound and histopathological findings.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** Self-funding

**References**


Physiological Study Comprising the Sequelae of Magnetic Radiation on Human

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Abstract

The sequelae of magnetic radiation of the towers of cellular phones were evaluated in this study depending upon a random social volunteer human male samples of ages 25 – 50 years and they were dwelling in houses close to the towers of cellular phones of distances not more than 150 miters far. The samples of these people were labelled as “Subjected”. Seventy five samples of blood were gained on a base of ages as twenty five from ages 25 – 30, twenty five from ages 35 – 40, and twenty five from ages 45 – 50 in addition to seventy five samples of people who did not dwell close to the towers and of the same previous pattern of ages and their samples were labelled as “Non subjected”. The results have demonstrated a significant elevation in transaminases (AST, ALT), blood calcium (Ca++), blood potassium (K+), total serum cholesterol (TSC), triacylglycerols (TAGs), very low density lipoprotein (VLDL), and low density lipoprotein (LDL).

Keywords: Magnetic waves, Radiation, Lipids.

Introduction

In our recent life there is an increasing need for the use of cellular phones without the ability to get rid of them taking into our mind that these phones operate on base of radiofrequency emitting and receiving ranging from 400 to 2000 megahertz (1). We know that the radiofrequency usage is not limited to the phones but it is also comprised in the medical appliances and therapy use (2). The stress effects on different body systems is well known and the same like occur when the body is subjected to radiofrequency due to impact energy absorption which could afflict the cellular membranes structures and cause sever damages to various cellular and subcellular structures and body organs like the effects on glands such as pituitary, adrenal, hypothalamus and others and you know this will afflict the functions of the body as a whole (3). Lipid profile, liver antioxidant enzymes, hepatic enzymes, renal functions, neuronal milieu, and others all are subjected to the risk of radiofrequency (4, 5, 6).

Materials and Method

Specimens’ collection

The specimens of blood were gained randomly from social volunteer human males of ages 25 – 50 years who were dwelling in Baghdad governrate close to the towers of cellular phones of distances not more than 150 miters far. The samples of these people were labelled as “Subjected”. Seventy five samples of blood were gained on a base of ages as twenty five from ages 25 – 30, twenty five from ages 35 – 40, and twenty five from ages 45 – 50 in addition to seventy five samples of people who did not dwell close to the towers and of the same previous pattern of ages and their samples were labelled as “Non subjected”. Once blood samples were collected as 5 ml/ people by the use of disposable syringes, they were poured into gel tubes to get blood serum to accomplish the necessary study parameters.

Study parameters

Special kits and a UV spectrophotometer (Apel – PD 303 UV, Japan) were used to perform all the study parameters.

► Total Serum Cholesterol, TCH (mg/dl)
Total serum cholesterol (TSCH) was estimated by using a special kit (Spinreact/CHOD – POD, SPAIN) according to the method of (7).

► Low density lipoprotein, LDL (mg/dl)

Serum LDL was obtained according to the formula: LDL-C = TC – HDL-C – TAG/5 (8).

► High density lipoprotein, HDL (mg/dl)

This was measured by using a chemical kit (HDL-Cholesterol (PTA) / Biolabo SA, France) according to (9).

► Serum very low density lipoprotein-cholesterol, VLDL (mg/dl).

Serum very low density lipoprotein was calculated by method of (8). VLDL = TAG / 5VLDL (mg/dl).

► Triacylglycerols (TAGs)

Triglycerides (TGs) are estimated by using a chemical kit (Triglycerides (GPO) / BIOLABO SA, France), depending upon the method of (10, 11).

► Blood Calcium level (mg/dl)

Blood Calcium level was estimated by the use of a special kit (Biomaghreb) according to method of (12).

► Blood Potassium level (mEq / l)

Blood Potassium level was estimated by the use of a special kit (Cypress Diagnostics) based on the method mentioned by (10, 13).

► Serum transaminases activity determination (Unit/ml)

ALT and AST enzymes were determined by the use of a special kit (Biomerrioux, Lyon-France) according to method of (14).

► Statistical analysis

Anova tests was depended to find the least significant differences (LSD) among groups by the use of SPSS version 21 program. Numbers in tables represent the mean ± standard deviation.

Results and Discussion

It is obvious when looking at the results (tables, 1 and 2) that all the human ages groups when continuously subjected to the radiofrequency of cellular phone towers have shown a clear significant elevations in serum AST, ALT, TSCH, LDL, VLDL, K+, and Ca++ besides a significant declination in serum HDL comparing them with the values of people who were not continuously subjected to the radiofrequency of towers at (P ≤ 0.05).

Dyslipidemia might be caused by obesity or due to consuming medications (15) and it was found that any disturbance in lipid metabolism could result in vascular pathological diseases (16). Exposure to magnetic waves could result in different stages of peroxidation of lipids and formation of reactive radicals like oxygen reactive radicals and nitrogen ones and both of them besides the peroxidation could affect the different cellular compartments and affect also the lipid metabolism and carrying vehicles (17, 23). The hepatic enzymes AST and ALT are considered as markers of stress and destruction of different body tissues since they are formed also by another boy regions or tissues like muscles, kidney, and heart (18, 19). It was found that the exposure to radiofrequencies could result in elevations in cortisol which is a predisposing of stress besides their effects on redox cycles and peroxides production and hence the radiofrequencies cause the elevations of AST and ALT (22). The elevations of blood calcium and potassium could result also by the frequency exposure as that these ions of great equilibrium in intracellular and extracellular compartments and they are carrying positive charges making the attracted to the negatively charged cellular membranes hence the disturbances caused by radiofrequency to the cell membranes and the destruction of them making the affinity towards these ions to be declined and much more ions could escape to extracellular compartments making them elevated in serum (20, 21).
Table (1). Human hepatic enzymes and electrolytes are affected by radiofrequency of mobile towers

<table>
<thead>
<tr>
<th>Ages (Years)</th>
<th>Groups</th>
<th>ALT (Unit/ml)</th>
<th>AST (Unit/ml)</th>
<th>Ca++ (mg/dl)</th>
<th>K+ (mEq / l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 - 25</td>
<td>Non subjected</td>
<td>b 46.4 ± 10.8</td>
<td>b 39 ± 12.8</td>
<td>b 9.5 ± 2.8</td>
<td>b 4.7 ± 2</td>
</tr>
<tr>
<td></td>
<td>Subjected</td>
<td>a 63.6 ± 15.1</td>
<td>a 75.1 ± 10.7</td>
<td>a 109.4 ± 20.8</td>
<td>a 9.8 ± 2</td>
</tr>
<tr>
<td>30 - 35</td>
<td>Non subjected</td>
<td>b 44.5 ± 5.4</td>
<td>b 31.1 ± 6.4</td>
<td>b 10.7 ± 3</td>
<td>b 4.7 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>Subjected</td>
<td>a 63.8 ± 8.5</td>
<td>a 78.5 ± 8.6</td>
<td>a 107.3 ± 20.4</td>
<td>a 8.2 ± 3.6</td>
</tr>
<tr>
<td>40 - 45</td>
<td>Non subjected</td>
<td>b 44.5 ± 5.4</td>
<td>b 32.1 ± 7.4</td>
<td>b 11 ± 2.7</td>
<td>b 4.5 ± 3</td>
</tr>
<tr>
<td></td>
<td>Subjected</td>
<td>a 69.5 ± 12.4</td>
<td>a 80.7 ± 12.5</td>
<td>a 109.5 ± 22.7</td>
<td>a 8 ± 3.9</td>
</tr>
</tbody>
</table>

LSD 10.7 35.1 97.3 3.16

Table (2). Human lipid profile is affected by radiofrequency of mobile towers

<table>
<thead>
<tr>
<th>Ages (Years)</th>
<th>Groups</th>
<th>TCH (mg/dl)</th>
<th>TAGs (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>VLDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 – 25</td>
<td>Non subjected</td>
<td>d 147.5 ± 8.7</td>
<td>d 127.5 ± 8.9</td>
<td>c 97.5 ± 8.9</td>
<td>c 24.1 ± 1.8</td>
<td>d 25.7 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>Subjected</td>
<td>b 340.2 ± 22.5</td>
<td>b 225.2 ± 21.7</td>
<td>b 163.5 ± 18</td>
<td>a 137.5 ± 3.3</td>
<td>b 45.2 ± 4.5</td>
</tr>
<tr>
<td>30 – 35</td>
<td>Non subjected</td>
<td>d 168.5 ± 17</td>
<td>d 138.7 ± 16.2</td>
<td>c 100.6 ± 7.7</td>
<td>c 39.7 ± 22.5</td>
<td>d 27.6 ± 3.4</td>
</tr>
<tr>
<td></td>
<td>Subjected</td>
<td>a 380.6 ± 21.3</td>
<td>a 267.2 ± 42</td>
<td>a 179.7 ± 16.2</td>
<td>a 152.6 ± 29.2</td>
<td>a 53.2 ± 11</td>
</tr>
<tr>
<td>40 – 45</td>
<td>Non subjected</td>
<td>d 162.8 ± 11.7</td>
<td>d 143.8 ± 17.2</td>
<td>d 59.5 ± 10</td>
<td>b 77.3 ± 10.2</td>
<td>d 28.6 ± 3.3</td>
</tr>
<tr>
<td></td>
<td>Subjected</td>
<td>c 297.1 ± 28.3</td>
<td>c 185.6 ± 6.9</td>
<td>c 99.7 ± 12.3</td>
<td>a 159.2 ± 39.6</td>
<td>c 35.5 ± 2.5</td>
</tr>
</tbody>
</table>

LSD 27.3 38.4 16.1 21.6 6.7
Conclusion

beside a significant decline in high density lipoprotein (HDL) of all Subjected samples as compared with the Non subjected ones at (P≤0.05); effects on redox cycles and peroxides production and hence the radiofrequencies cause the elevations of AST and ALT, also The elevations of blood calcium and potassium could result also by the frequency exposure as that these ions of great equilibrium in intracellular and extracellular compartments.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

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Assessment of Transdermal Vasodilatatory Effect of a Combined Panthenol, Amlodipine, Isosorbide Dinitrate and Betahistine HCl on Peripheral Vessels

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Abstract

Peripheral vascular diseases are group of disorders characterized by stenozing peripheral circulation as a complication of primary disease like diabetes mellitus. Peripheral vascular diseases affect 202 million around the world. Peripheral vascular complications of diabetes mellitus are common which affect 30 million over the world and may give rise to infectious necrotizing sequelae called diabetic foot. In a trial of assessment for transdermally applied vasodilator drugs, 30 individual had participated in a case controlled study. Test group (N =11) and control group (N =19) were assessed for the signs of vasodilatation over the dorsum of the hand and figure blood perfusion detection. There was significant increase in perfusion index (from 7 to 11) induced by the test vasodilator as compared with control (from 7 to 8) P <0.05 and parallel results were obtained in induction of redness (redness ratio of 7 with C.I. over 0.95 and thermal increase in degrees of (C) over the dorsum of the hand in comparison with the control group. From the overall results the combined vasodilatatory transdermal formula caused a significant peripheral vasodilatation which could be a candidate therapeutic effect in diabetic foot.

Key words: PVD; combined transdermal formula; peripheral blood perfusion; thermal effect; nitrate; RGB.

Introduction

Peripheral vascular disease (PVD) is the abnormality of vessels located outside of the heart and brain, mainly leg vessels characterized by complete or partial blockage and impairment of perfusion. PVD could affect both arteries (peripheral arterial disease PAD) and veins. PVD is one of the significant health challenges that affects up to 20% after the age of 60 year (1,2) and 202 million patients around the world (3,4).

Different etiologic and pathogenic factors might share common impacts on peripheral circulation. Of the most critical form of PVD presentation is the angiopathic complications of diabetes mellitus (5-7).

However, Burger disease and Raynaud phenomenon are also characterized by impaired peripheral circulation (8,9). Impaired peripheral circulation in uncontrolled diabetes is characterized by different pathological processes including occlusive ischemia (10-12), impaired immune response and peripheral neuropathy (13,14). Impairment of circulation and immune system in addition to hyperglycemia and neuropathy can severely deteriorate any skin lesion in the foot. That lesion may be refractory to treatment due to lacking of pharmacokinetic opportunity of drugs treatment to diffuse to extreme tissues of the lower limbs. This pathogenic fact make foot prone for untreatable infections that even cause ascending cellulitis, tissue gangrene and septicemia which is frequently necessates amputation of the foot or lower limb (15).

A potent systemic vasodilatatory approach like nefidipine, nicorandil can cause intolerable adverse effects like palpitation, throbbing headache and edema due to the need for extensive vasodilatation (16-18). Transdermal route of application gives opportunity for rational option of applying combined efficacious
vasodilators just proximal to the site of lesion that bypassing systemic impacts (19-21).

Different methods are used to assess peripheral circulation. These include: Doppler ultrasound, angiographic techniques in addition to the clinical assessment of peripheral pulsation. However, other methods like infrared detection of blood flow. Quantitative assessment of peripheral perfusion is detected by the perfusion index PI which ranges between 0.02% to 20% according to site whereas blood flow is 5000 ml/min (22,23). Tissue blood flow rate varies also according to type of tissue. Thermographic camera images are also reliable noninvasive methods for long term follow up of circulatory perfusion (24-26).

In the present work, the effect of transdermally applied combination of panthenol, mineral oil base, amlodipine, pH buffer, isosorbide dinitrate, stabilizer and betahistine with peripheral perfusion rate and thermographic analysis were assessed.

Sample, Materials and Method

A 40 individuals (20f/20m) aged 20-24 years (body weight 50-65 kg) have participated in clinical assessment of the effects for the designed formula.

Groups of the study:
1- Test group (N = 15: 8 f/ 7 m).
2- Control group (N = 25: 12 f/ 13 m).

All persons had no chronic medical illnesses.

Study design

The study was arranged in a pilot clinical trial design to determine the effects of test formula so that one group had transdermally applied vasodilators and the other had only the blank application.

Medical and Research ethics requisites

These considerations were fulfilling Geneva requisites guide for medical ethics. All of the participants were informed about the study design and the expected topical adverse effects of the agents used.

Free autonomy, drugs safety, rights reservation, individual consents and drugs benefits were considered and insured.

Materials used

All the materials used were processed, filtered, and confirmed with Fourier UV/V range 200-700 nm based on British Pharmacopia 2007 drugs standard spectrophotometry.

Drug dosages were weighed for base of transdermal formula design.

- Amlodipine ( Bristo, UK, production vs expiry:PD-ED: 2013-2016)
  7000 mg package. Used in 3% within transdermal design.

  100 mg package. Used in 0.05%.

  Used in dosage of 0.3%.

  Used in 0.02%.

- Hydrocarbone base vehicle.

Mineral laurate, stearate and solid excipients were prepared for 20 ml per dosage form.

Treatment Mode

Objective vasodilatation parameters were measured prior to application of both blank and test formula as a
Methods of assessment of vasodilatation

Peripheral blood perfusion was measured with Beijing Safe Heart Technology. Transducer was connected to PERFUSION-Kufa program of analysis developed by Dr. Hussein Abdulkadhim on Mathwork 2013a blockset for estimating model formula of perfusion in response to the transdermal test treatment.

Perfusion index PI is readily detected and calculated by the computer from which another measurement could be estimated which is the perfusion range is determined with in unit of time to calculate the rate of perfusion in ml/min and compared for the control and observational control group. The fixed level of the hand and index figure was carefully considered because it is important confounder.

Another assessment method for vasodilatatory activity of the test formula includes thermographic correlative analysis detected by combined spot tissue thermal camera.

Since temperature correlates proportionally with rate of peripheral blood perfusion, a rise in temperature of the dorsum of the hand correlates with a parallel rise in the rate of perfusion (parameters detailed in guidelines manual).

Image processing program was used to analyze RGB shift as an indicator of redness associated with vasodilatation by the test combination.

Lab techniques quality confirmation:

Accuracy of Safe Heart Technology was X +/- 0.2 PI. Efficiency of the thermographic camera was insured by software processing and calibration.

UV/V (Cecil, UK, Programmed wavelength, accuracy: X +/- 0.5%). Sensitive Balance (mini digital, China, accuracy: X +/- 0.01 mg). PHELECT, USA computerized pH measuring electrode is used to assess adjustment of formula pH around 7.5.

The Sample Size, Statistical Processing and Analysis

The sample size for this study was calculated based on the Cochran formula n= z^2 * p*q/e^2 where (e) is the margin error, p and q are the complimentary proportions and z is the score at confidence level 95% equals to 1.96. Although the margin error will be wide, however it’s preferred to reduce the sample size as much as it’s statistically possible in a pilot clinical trial in order to fulfill FDA approval guidelines where a sample size of 10 individuals is considered while conducting a phase zero trial.

Mathwork 2013a model interpolation was used to verify the perfusion curve. Perfusion index PI modification ratio was estimated with C.I. of 0.24 and a confidence level of 95%. Sample size was determined based on Cochran formula. Paired t test with Minitab 2014 at P <0.05.

Results

Calculation of the rate of perfusion.

![Figure 2](image-url): Matlab analysis of peripheral perfusion rate by model analysis and rate determination. The amplitude axis was calibrated to obtain perfusion rate in mL/min.
Figure (3): The mean perfusion index PI (normalized % +/- SD) taken from hand index finger for persons taking the test transdermal formula (raised from 7 +/- 2 to 11 +/- 2) as compared with the control (7 +/- 2 to 8 +/- 2 PI).

Figure (4): The objectively analyzed RGB to determine level of redness induced on the dorsum of the hand by the combined vasodilator formula after 10 minutes from topically being applied as a quantitative indicator of vasodilatatory effect.

Figure (5): The effect of the test formula on the RGB right shift of red intensity in comparison with the control. Red intensity shift was from 234 to 256 as compared to control which caused just a shift from 240 to 243.
Discussion

The sample of this study represented healthy nondiabetic population. It was taken as a model to assess the vasodilatation at the upper extremities where temperature, RGB and peripheral perfusion were measured from.

That sample was beneficial to estimate the vasodilatatory prior to recommendation in diabetic patients’ sample since this was in agreement with FDA phases of drug evaluation.

Transdermal approach is a promising way of treatment since many advanced techniques had made this route more reliable and superior to conventional systemic administration of drugs. Of these advances techniques are the nanoparticulate reservoir of active drug, sonophoresis, electrophoresis, microarray needle and matrix patches (27).

Transdermal application of drugs has many advantages like convenience, safety and it is easily controlled.

The results showed that a significant increase in perfusion index (PI) was obtained with the test formula (from 7 to 11%) in comparison with the blank base (from 7 to 8%) with ratio of increase 1.3 in PI. In one study, amlodipine showed a significant induction of increased forearm blood flow. Results of some studies concerned assessment of vasodilatory effect of transdermal amlodipine monotherapy have agreed with this current study (29). Panthenol in combination with other additives was used as a topical wounds healing enhancer (29). Betahistine effects in some applications agreed with this study (30), however there was no improvement of cold regional pain syndrome by using transdermal isosorbide dinitrate in a small controlled trial (31,32).

Redness parameters have confirmed and went parallel to data of perfusion rate increment. The mean RGB was raised from 234 to 256 in test formula group as compared with a mild raise from 240 to 243 in blank taken group. This indicates a redness ratio of 7 with C.I. at 0.95 (33).

Thermographic results revealed parallel findings with redness image processing outcomes. Hand temperature was raised from 36.0 to 37.8 ºC in vasodilatory test group in comparison with 35.5 to 36.1 ºC in the control. The temperature raising activity was 3 and C.I. at 0.95.

Conclusion

From the overall effects, the combined transdermal formula panthenol, mineral oil base, amlodipine, pH buffer, isosorbide dinitrate, stabilizer and betahistine showed a significant vasodilatory effects on the peripheral circulation. There was a significant increase of thermal effect due to the topical combination of vasodilators in comparison with the control.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

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Evaluation of Using Titanium Mesh in the Reconstruction of Traumatic Orbital Floor Fracture

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Abstract

Background: Among the nonresorbable implants, Titanium mesh is the most common graft used for orbital reconstruction. It is continuously improved to achieve accurate restoration of orbital volume. To evaluate the using of Titanium mesh for the reconstruction of traumatic orbital floor fractures.

Patients and Methods: A total of (32) patients were enrolled in this study where operated under general anesthesia to repair the orbital floor fractures by using titanium mesh, and they were (24) males and (8) females. (16) patients had Enophthalmos,(10) patients had Diplopia,(6) patients had both enophthalmos with diplopia; all patients had ecchymosis, Subconjunctival hemorrhage, Parasthesia of infraorbital nerve.

Results: The results were well represented as the following: (29) patients (90.62%) had no both diplopia & enophthalmos. Postoperative complication had been found in only (3) patients (9.3%), Two patients (6.25%) had diplopia, one patient (3.1%) had Enophthalmos.

Conclusions: Titanium mesh has a long track record of reconstruction of large orbital floor defects and correction of globe malposition.

Keywords: Titanium mesh; orbital reconstruction and fracture

Introduction

Orbital floor fractures have specific clinical attention for many reasons. Failure to recognize and treat them early may result in severe sequelae. However, despite surgical intervention, orbital floor fractures are associated with the risk of persisting sensibility disorders, enophthalmos, and permanent diplopia. (¹) The choice of the ideal material for reconstruction of orbital floor and walls remains highly controversial. Many materials, from different sources, have been described for that task. The ultimate goals are the reconstruction of the bony orbital defect with restoration of anatomy, volume, function, and esthetics. Each type of material has advantages and disadvantages, but the most important characteristic of a material is to allow those surgical objectives to be achieved. (²) Titanium mesh is the most common grafts used for orbital reconstruction because of its biocompatibility, availability, rigid fixation and no donor site needed. (³) There is still controversy existing about the ideal material for orbital reconstruction; autogenous and synthetic materials have been used for many years, and both of them have merits and demerits. The common sources of autogenous graft are the calvarium (⁴), rib, iliac crest, and auricular or nasal septal cartilage. Sakakibara et al. (⁵) used the iliac cancellous bone of only 1 mm thickness for the reconstruction of the orbital floor. Numerous synthetic materials have been developed with the advantages of availability, no donor site morbidity, and decreased operation time. The choice of material for reconstruction is largely determined by the experience of the surgeon and implant cost. Among the nonresorbable implants, titanium mesh and Medpor (⁶) are the most common grafts used for orbital reconstruction. Both materials are being
continuously improved to achieve accurate restoration of orbital volume. During the past several decades, the standard of care for orbital reconstruction after trauma has been autogenous bone grafts, complications of bone grafts, including donor site morbidities such as scar alopecia and graft resorption with delayed enophthalmos, have inspired an interest in the use of alloplastic substitutes such as titanium, titanium’s role in orbital reconstruction was limited originally to small orbital defects, and as an adjunct to bone grafts, more recently, clinical studies have documented the sole use of titanium mesh to reconstruct large orbital defects. (7) Orbits reconstructed with titanium mesh show better results than those reconstructed with bone grafts. (8-10)

Titanium is a chemical element with symbol Ti and atomic number 22. It is a lustrous transition metal with a silver colour, low density, and high strength. (11) Titanium can be alloyed with iron, aluminium, vanadium, and molybdenum, among other elements, to produce strong, lightweight alloys. (11) The two most useful properties of the metal are corrosion resistance and the highest strength-to-density ratio of any metallic element. (12) In its unalloyed condition, titanium is as strong as some steels, but less dense. (12) Titanium is used in steel as an alloying element (ferro-titanium) to reduce grain size and as a deoxidizer, and in stainless steel to reduce carbon content. (11) Titanium is often alloyed with aluminium (to refine grain size), vanadium, copper (to harden), iron, manganese, molybdenum, and other metals. (13)

Medical applications: Because it is biocompatible (it is non-toxic and is not rejected by the body), titanium has many medical uses, including surgical implements and implants, such as hip balls and sockets (joint replacement) that can stay in place for up to 20 years. (13) The titanium is often alloyed with about 4% aluminium or 6% Al and 4% vanadium. (14) Because titanium is non-ferromagnetic, patients with titanium implants can be safely examined with magnetic resonance imaging (convenient for long-term implants). The features of 3D Titanium mesh are: malleable, 0.3mm thickness, 1-1.3mm screws, available for right and left sides. (15) Advantages of titanium mesh plates: (15)

1. availability, biocompatibility
2. ease of intraoperative contouring
3. rigid fixation
4. Radiopacity
5. No donor site needed
6. Tissue incorporation may occur

Disadvantages:
1. Irregular edges of the mesh may catch prolapsed orbital fat. (16)
2. Difficulties with ease of insertion
3. Difficult to remove if required

Complications of titanium mesh:
1. While a nonresorbable material, titanium cannot be replaced by new soft tissue or bone tissue and remains in situ indefinitely. This may cause possible late side effects, including toxicity due to metal ion release. (17)
2. The fibrous reaction between the implant and the orbital contents caused the eye movement restriction and the lid retraction. (18)
3. To avoid adherence syndrome, titanium mesh plates should be placed 2 mm away from the orbital rim. (18)

**Patients and Methods**

A total of (32) patients were enrolled in this prospective study was conducted from 2014 to 2016. There were (24) males and (8) females. Patients’ age ranged between (10-50) years. The mean age was 30 years. Inclusion criteria: Patients were included in this study according to the following criteria: Patients with orbital floor fractures regardless of their age or gender and type of missile injury (Blast or Bullet), Orbital floor defect (small to very large defects) confirmed by C.T. scanning, Patients with Enophthalmos and Patients with Diplopia due to mechanical obstruction. Exclusion criteria: Patients with the following criteria were excluded from the study: Serious general disease and unfit for surgery, Refused to participate, All orbital fractures regardless of the fracture site and Patient with diplopia due to neural causes.

**Measuring of Enophthalmos:**

The mean preoperative enophthalmos was about (4) mm measured by a ruler from the lateral orbital rim of the injured eye to the most anterior projection of the
globe in comparison to the intact contra-lateral eye.

Results

Sex and age of the patients:

There were (24) males (75%) and (8) females (25%) with a male to female ratio of (3:1), (Figure 1 and 2). The age distribution of the patients revealed a mean age of (30 ± 2.1) years furthermore, {2} patients (6.25%) aged (10-19) years, {10} patients (31.25%) aged (20-29) years, {8} patients (25%) aged (30-36) years, {7} patients (21.875%) aged (37-45) years, {5} patients (15.625%) aged (46-50) years.

Figure 1. Gender distribution of the patients, (N=32, Male to female ratio; 3:1) Figure 2. Age distribution as percentages of the studied group, (N=32)

Postoperative Complications

As shown in table 1, fortunately.

1. (29) patients (90.625%) had no complications,
2. Post-operative complication had been found in only (3) patients (9.375%).
3. Two patients (6.25%) had diplopia, 3. one patient (3.125%) had Enophthalmos and
4. No patient (0%) had extrusion.
Table 1. Post-operative complications of patients.

<table>
<thead>
<tr>
<th>Complication</th>
<th>No. of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diplopia</td>
<td>2</td>
<td>6.25</td>
</tr>
<tr>
<td>Enophthalmos</td>
<td>1</td>
<td>3.125</td>
</tr>
<tr>
<td>Extrusion of titanium mesh</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No complications</td>
<td>29</td>
<td>90.625</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>100</td>
</tr>
</tbody>
</table>

Discussion

Age Distribution

In the current study, the highest risk group were the young patient (20-29 years) represented as (31.25%) and this was in agreement with Leibsohn et al., 1976 (19); Greenwald et al. 1979 (20); Crumley et al. 1977 (21); Andersen et al. 1985 (22). In fact that this age group represents the time of maximum activity in human life, especially males.

Sex Distribution:

Twenty four patients (75%) were males, while 8 patients (25%) were females. The predominant male to female ratio (3:1) can be explained by the fact that males spend most of their time outdoors, and due to occupational and recreational preferences. This result is consistent with the findings of the studies by: Thomas 2005 (23), Michael 2000 (24), David 2004 (25), Gordon 2004 (26), Joe 2005 (27), Geoffrey 2005 (28), Petrus 2006 (29).

Reconstruction material:

The orbital wall is one of the most frequently damaged parts of the maxillofacial skeleton after midfacial trauma. Regardless of the fracture site, blow-out fractures can cause various functional and aesthetic sequelae. Preventing these complications from becoming long-term problems is very important, and it depends strongly on the materials used for bridging the orbital wall defects. The prerequisites of an ideal material are good biocompatibility, easy to manipulate and insertion, and it should allow fixation to the host bone by screws, wire, or sutures. It should be cheap, readily available, and strong mechanical strength to support the orbital structure. Titanium mesh has a long track record in the reconstruction of large orbital defects and correction of globe malposition. The advantages of titanium mesh plates are availability, easy intraoperative contouring, and rigid fixation. According to our work, we found it can be adapted to complex structures easily, and it can also be cut to shape as well. The orbits reconstructed with titanium mesh showed better overall reconstructions than those reconstructed with bone grafts, and according to our work, we agree with Ellis E 3rd(1), Tan Y 2003 (8).

Complications

Pre-operatively enophthalmos occur in (16) patients (50%), diplopia occur in (10) patients (31.25%), and (6) patients (18.75%) had both enophthalmos and diplopia. After reconstruction, we have only one patient who has persistent enophthalmos presented with extensive injury to surrounding bony structures with loss of bony architecture. We agree with the finding of { Saikrishna Degala, 2012 (30)}.

Postoperatively, diplopia occurs in two patients, mainly in the upward gaze. This consistent with that reported by Amrith S et al. 2000 (31). Where they reported that diplopia is not uncommon postoperatively, it is typically only disturbing when occurring in the primary or downward gaze. However, titanium plates are permanent foreign bodies. Several late-onset complications related to the titanium mesh plate have been reported, such as infection, extrusion, implant migration, residual diplopia, etc. (32). But we found in our study, the postoperative clinical and radiographical examination verified the anatomical reduction of the orbital floor. There was no displacement or resorption of the orbital floor or loosening or extrusion of the screws or mesh (6-9) months after the operation, and no modifications in the visual acuity compared with autologous materials have several disadvantages, including high risk of nerve and blood vessel injury, donor site morbidity, cosmetic disturbance, minimal controllability and an unpredictable degree of absorption (33,34).

Conclusions

1. Missile trauma was the most frequent cause of orbital injury followed by road traffic accidents, sports injury, and falls from height.

2. Titanium mesh has less complication postoperatively, such as infection and migration of mesh.

3. This study highlights the ability of the
alloplastic mesh to satisfactorily correct post-traumatic orbital sequelae, including enopthalmos and diplopia.

4. Titanium mesh can be considered to be the excellent orbital floor repair material.

5. No extrusion of Titanium mesh or rejection by the host was seen in the studied group.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

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Association of Epstein- Barr Virus (EBV) with the Development of Nasopharyngeal Carcinoma (NPC) in Western Region of Iraq: Unmatched Molecular Case-Control Study

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Abstract

Background: Nasopharyngeal Carcinoma (NPC) is uncommon in Iraq, but its incidence is raising due to increased exposure to diverse risk factors. Many of the NPC-related risk factors are becoming more and more apparent in Iraq. The exactly risk factors for nasopharyngeal carcinoma (NPC) in Anbar province, Iraq are not known.

Objectives: To determine the association between Epstein- Barr virus (EBV) infections and other risk factors with the development NPC of Iraqi patients.

Patients and Method: Sixty-seven paraffin-embedded tissues of NPC cases, 134 normal noncancerous nasopharyngeal biopsy samples, and tonsillectomy specimens from patients with chronic hypertrophic tonsillitis as controls were enrolled in the study that was conducted between 12 January 2012 and 21 January 2019. DNA of EBV was extracted from both controls and neoplastic tissues and analyzed by PCR technique using primers specific to EBV Latent Membrane Protein-1 Oncogene (LAMP-1) for the presence of EBV. A Questioners data form for all patients and controls were filled by the researchers regarding other risk factors of NPC including the patient’s age, sex, residence, radiation exposure, history of chronic rhinitis, family history of NPC, tobacco smoking, alcohol consumption, herbal medicines, tea consumption, exposure to formaldehyde and exposure to different inhalants.

Results: The following risk factors were found to be independently associated with illness: EBV (adjusted odds ratio [OR] 7.852, 95% confidence interval [CI] 2.22–27.72), herbal medicine (OR 19.051, CI 7.56–47.95) and Family history of NPC (OR 63.717, CI 6.67–607.96).

Conclusion: Combination of family history of NPC , EBV exposure and herbal medicine was a strong risk factor for NPC.

Keywords: Epstein - Barr virus, Nasopharyngeal Carcinoma, PCR, EBV-LMP-1, other risk factor, case-control study

Introduction

Nasopharyngeal carcinoma (NPC) is a human malignancy derived from the epithelium of the retro-nasal cavity and it is one of the most frequent head and neck cancers with elevated prevalence rates in Asia and western North Africa (1, 2) with, approximately 86691 incident cases of NPC and 50 831 NPC-related deaths in 2012 worldwide (3, 4).

Previous studies demonstrated Epstein –Barr virus (EBV) infection, genetic susceptibility, diet, chromosomal disorders, aberrant promoter hyper methylation and other genetic related factors and other environmental exposures (1, 5, 6), Cigarette smoking, alcohol consumption and Consumption of salted (1, 7, 8), Family history of NPC (1, 9). The present study to know the roles of EBV with
other Risk factor in development of NPC.

Materials and Method

Study design

Case-control sets were included for analysis, provided that two control was matched for each case by age, sex and general geographical location of the case’s residence. A standard questionnaire for all patients and controls were filled by the researchers regarding other risk factor of NPC. Including the patient’s age, sex, residence, Radiation exposure, history of chronic Rhinitis, family history of NPC, tobacco smoking with alcohol consumption, herbal medicines, Tea consumption, Exposed to formaldehyde and, exposure to different inhalants. This study was conducted in the Anbar province and its neighbor area and biopsies were collected over a 7 years period between 3 January 2012 and 1 January 2019. The protocol was approved by the Microbiology Department, Anbar medical College. All patients provided informed consent for participation in the study and for biopsy samples taken from the tumors and tonsillectomy specimens.

Molecular study of EBV

Patients and Methods:

Sixty-seven paraffin-embedded tissues of NPC cases and 134 normal noncancerous nasopharyngeal biopsy samples and tonsillectomy specimens from patients with chronic hypertrophic tonsillitis as controls were enrolled in the study that was conducted between 3 January 2012 and 1 January 2019 to investigate the presence of LMP-1 gene. Paraffin-embedded tissues of NPC cases were selected from the archives of private pathology laboratories in Anbar province and cases were classified according to the WHO classification (10). Archived slides were reviewed by two pathologists for confirmation of diagnosis and tissue adequacy for extracting of DNA. Controls were matched on gender, residence and year of birth. The molecular analyses were carried out at private laboratories in Baghdad city.

Tissue Processing, PCR Amplification and Gel Electrophoresis

The tissue samples were purified from archival paraffin embedded tissue blocks as described previously (11). DNA of EBV was extracted from both cases and controls using the same procedure described previously (12) and PCR amplification for detection of EBV-LMP1 gene were processed through using oligonucleotide (sense BN1, antisense BN2) as follow (sense BN1: 58-AGC GAC TCT GCT GGA AAT GAT-38 or antisense BN2: 58-TGA TTA GCT AAG GCA TTC GCA CCT CCA-38) as described previously (13). The products were then examined on 1.5% agarose gel electrophoresis in 1× Tris-boric acid–EDTA (TBE) solution and stained with ethidium bromide to verify the presence of 316 bp PCR product.

Statistical Analysis

A matched design and a case-control ratio of 1:2 was selected as the most appropriate strategy in order to maximize the study power. To detect an association with a matched odds ratio of 2.0 at the 5% significance level with 80% power (assuming 20% exposure level among controls), a sample size of 67 cases and 134 controls was required (14).

Epi Info Version 7.02 (15) and SPSS version 24 were used to calculate crude matched odds ratios (OR) with 95% confidence intervals (CI) and two-tailed P-values to estimate the association between various potential risk factors and NPC. Following the univariate analysis, SPSS were used to calculate adjusted odds ratios by conditional binary logistic regression of risk factors with a P-value < or = 0.25.

Results

During the study period, 67 NPC cases were collected and 134 normal lymphatic tissues registered as possible controls. From the total number of controls registered, 134 were included in the study, 67 cases matched to 2 controls. The demographic characteristics of the cases and controls are shown in Table 1. More males than females were enrolled in the study, with the majority of subject’s in age group 19 – 38 (40.3%) and 58 years old and over (49.3%). Regarding residence, rural patients more than urban.
Table 1: Demographic characteristics of subjects

<table>
<thead>
<tr>
<th>Character</th>
<th>NPC Cases N= 67 (%)</th>
<th>Healthy Control N= 134 (%)</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>50 (74.63%)</td>
<td>100 (74.63%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Female</td>
<td>17 (25.37 %)</td>
<td>52 ( 25.37 )</td>
<td></td>
</tr>
<tr>
<td>Residence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>42(62.69 %)</td>
<td>84 (62.69 %)</td>
<td>1.000</td>
</tr>
<tr>
<td>Urban</td>
<td>25 (37.31 %)</td>
<td>50 (37.31 %)</td>
<td></td>
</tr>
<tr>
<td>Age Group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;= 18</td>
<td>1(1.5%)</td>
<td>2 ( 1.5%)</td>
<td></td>
</tr>
<tr>
<td>19 - 38</td>
<td>27 (40.3%)</td>
<td>54 (40.3%)</td>
<td></td>
</tr>
<tr>
<td>39 - 57</td>
<td>6 (9.0%)</td>
<td>12 (9.0%)</td>
<td></td>
</tr>
<tr>
<td>58+</td>
<td>33 (49.3%)</td>
<td>66 (49.3%)</td>
<td></td>
</tr>
</tbody>
</table>

Detection of EBV LAMP1

EBV were Detected in NPC cases and healthy cases on Agarose gel electrophoresis stained with Rad safe as shown on Fig.1.

![Fig. 1: Detection of EBV in NPC cases and healthy cases on agarose gel electrophoresis stained with Rad safe. Lane M shows a molecular size marker. Lanes 1-15 shows the positive bands of EBV-wt-LMP1 with product size 316 bp.](image-url)
Univariate analysis of risk factors

Potential risk factors for NPC (i.e. those with a matched odds ratio > 1) are shown in Table 2. Infection with EBV (OR 10.631, CI 3.654-30.930), Herbal Medicine (OR 20.7955, CI 9.531-45.457), history of chronic Rhinitis (OR 3.792, CI 1.069-13.447), Family history of NPC (OR 74.233, CI 9.752-565.051) and Exposed to formaldehyde (OR 2.375, CI 1.303-4.330), were highly and moderate risky and significantly associated with NPC.

Cigarette Smoking (OR 1.309, CI 0.727-2.356) with a low risk of illness, but this was not statistically significant.

In regard to Alcohol drinking (OR 0.715, CI 0.381-1.342), High Background Radiation Areas (OR 0.662, CI 0.068-6.484), Tea consumption (OR 0.557, CI 0.307-1.010) and Dust and/or exhaust exposure/domestic fumes intake (OR 1.031, CI 0.569-1.870) were non significantly associated with NPC.

Insufficient data were obtained on Cannabis intake, Salted fish consumption and history of chronic respiratory tract conditions that may be assumed or potential risk factor for NPC, and as such, no conclusions could be made about these exposures.

Table 2: Univariate analysis of selected risk factors for NPC (matched OR > 1)

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>NPC Cases (N=67)</th>
<th>Healthy Control (N=134)</th>
<th>Matched Odds Ratio</th>
<th>95% CI</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBV Positivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>63 (94.03 %)</td>
<td>80 (59.70 %)</td>
<td>10.631</td>
<td>3.654-30.930</td>
<td>0.000</td>
</tr>
<tr>
<td>Negative</td>
<td>4 (5.97 %)</td>
<td>54 (40.30 %)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herbal Medicine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>45 (67.16 %)</td>
<td>12 (9.00 %)</td>
<td>20.7955</td>
<td>9.531-45.457</td>
<td>0.000</td>
</tr>
<tr>
<td>No</td>
<td>22 (32.84 %)</td>
<td>122 (91.00 %)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of chronic Rhinitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>7 (10.45 %)</td>
<td>4 (2.99 %)</td>
<td>3.792</td>
<td>1.069-13.447</td>
<td>0.044</td>
</tr>
<tr>
<td>No</td>
<td>60 (89.55 %)</td>
<td>130 (97.01 %)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family history of NPC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>24 (35.82 %)</td>
<td>1 (0.75 %)</td>
<td>74.233</td>
<td>9.752-565.051</td>
<td>0.000</td>
</tr>
<tr>
<td>No</td>
<td>43 (64.18 %)</td>
<td>133 (99.25 %)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exposed to formaldehyde</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>36 (53.73 %)</td>
<td>44 (32.84 %)</td>
<td>2.375</td>
<td>1.303-4.330</td>
<td>0.006</td>
</tr>
<tr>
<td>No</td>
<td>31 (46.27 %)</td>
<td>90 (67.16 %)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cigarette Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>35 (52.24 %)</td>
<td>61 (45.52 %)</td>
<td>1.309</td>
<td>0.727-2.356</td>
<td>0.347</td>
</tr>
<tr>
<td>Nonsmoker</td>
<td>32 (47.76 %)</td>
<td>73 (54.48 %)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol drinking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>20 (29.85 %)</td>
<td>50 (37.31 %)</td>
<td>0.715</td>
<td>0.381-1.342</td>
<td>0.347</td>
</tr>
<tr>
<td>No</td>
<td>47 (70.15 %)</td>
<td>84 (62.69 %)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Background Radiation Areas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1 (1.49 %)</td>
<td>3 (2.24 %)</td>
<td>0.662</td>
<td>0.068-6.484</td>
<td>1.000</td>
</tr>
<tr>
<td>No</td>
<td>66 (98.51 %)</td>
<td>131 (97.76 %)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tea consumption</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>34 (50.75 %)</td>
<td>47 (35.07 %)</td>
<td>0.557</td>
<td>0.307-1.010</td>
<td>0.067</td>
</tr>
<tr>
<td>No</td>
<td>33 (49.25 %)</td>
<td>87 (64.93 %)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dust and/or exhaust exposure/domestic fumes intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>28 (41.79 %)</td>
<td>55 (41.04 %)</td>
<td>1.031</td>
<td>0.569-1.870</td>
<td>1.000</td>
</tr>
<tr>
<td>No</td>
<td>39 (58.21 %)</td>
<td>79 (58.96 %)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Multivariate analysis of risk factors

Of the ten risk factors included in the conditional binary logistic regression model, a significant independent association with illness was found for the following three risk factors: exposure to EBV (adjusted OR 7.852), Herbal Medicine using (adjusted OR 19.051), and Family History NPC (adjusted OR 63.717) (Table 3).

Table 3: Multivariate analysis of selected risk factors for NPC

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Adjusted odds ratio</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBV Positivity</td>
<td>7.852</td>
<td>2.224-27.720</td>
<td>0.001</td>
</tr>
<tr>
<td>Herbal Medicine using</td>
<td>19.051</td>
<td>7.568-47.953</td>
<td>0.000</td>
</tr>
<tr>
<td>Family History NPC</td>
<td>63.717</td>
<td>6.678-607.963</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Discussion

The results obtained from our study suggest a strong effect of exposure to EBV, herbal medicine using and family history of NPC on NPC risk. Exposure to formaldehyde and the history of chronic rhinitis were also related to NPC risk, whereas the exposure to high background radiation, cigarette smoking, alcohol drinking, tea consumption and dust and/or exhaust exposure/domestic fumes intake in Anbar province were not related to NPC risk even after adjusting for major risk factors of NPC. Epidemiological study have combined three suspected risk factors (EBV, herbal medicine using and Family History NPC) into a simultaneous analysis factors and study their possible synergetic effects, and reports of relatives with NPC using conditional multivariate logistic regression.

Therefore, it is strongly suspected that NPC risk is affected by cofactor(s) in addition to EBV infection like herbal medicine using and family history NPC were strong risk factors of NPC. This finding is consistent with that of Sriamporn et al.(1992) (16) who showed elevated VCA/IgA and neutralizing antibodies against EBV DNAse with a 20-fold increase in NPC risk for subjects seropositive for VCA/IgA antibodies, and a 30-fold increase for those seropositive for both biomarkers.

Our study agreed with two studies from Taiwan that showed high prevalence of the LMP1 DNA (94.7–100%) in swab samples from NPC patients but not from control groups and other study that demonstrated that NPC is well specimen that can demonstrate the transforming ability of EBV latent membrane protein which is expressed in approximately 65% of NPC tumors (5, 18, 19), So the plasma EBV-DNA level might be a sensitive and reliable biomarker for the diagnosis of NPC at a molecular level/clinical practice (20, 21) as EBV is a major etiologic factor for (NPC), and it is detected in tumor cells of virtually all NPC cases (22).

The results from Tables 2 suggest a synergetic effect between herbal medicine using and EBV. These results are in agreement with those obtained by West et al (23) that confirms a 2-4-fold excess risk of NPC in association with use of traditional herbal medicines through several case control studies. In contrast to other findings, however, no evidence of herbal medicine using links to NPC was detected (1). Herbal medicine using might be a strong risk factor of NPC through activation EBV in latent EBV infected cell or through a direct promoting effect on EBV-transformed cells (24).

Furthermore Family History NPC (indicated as a history of first degree relatives with NPC) was risky for NPC as approved by Busson et al, (2004) (5) who found there is evident that genetic factors might be of importance for the etiology of NPC. The mechanism of familial clustering is not understood and it may reflect genetic factors, shared environmental factors, or both (9). In accordance with the present results, previous studies have demonstrated that a strong association between our estimates of formaldehyde exposure and NPC (9).

The present study identifies cigarette smoking, Alcohol drinking, Tea consumption and dust and/or exhaust exposure/domestic fumes intake are statistically non-significant risk factors for NPC after adjusting these identified risk factors. These findings are in contrary to some studies that suggested these factors are risk factor for NPC. However, other studies which have suggested
that the relationship of NPC to these risk factors are less clear and inconsistent (1, 7-9).

To our knowledge, this study is the first study in Anbar province that illustrates the association between some risk factors including EBV and NPC development, hoping that a better understanding of the etiologic interactions between viral and environmental factors in the pathogenesis of NPC.

Conclusion

Combination of family history of NPC, EBV exposure and herbal medicine was a strong risk factor for NPC.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding: Self-funding

References


Molecular Detection of Toxogenic Cyanobacteria Isolated from Tigris River in Baghdad City –Iraq

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Abstract
Algae and their contamination are being increasingly reported worldwide that cause a serious hazard to environmental and human health. Cyanotoxin was the most algal toxin reported to be produce by several orders of cyanobacteria. In 2017 cyanobacteria were isolated from fresh water of Tigris River and identified by light compound microscope as well as conventional PCR. Five isolates of cyanobacteria which successfully amplified a gene fragment from the phycocyanin shared by all cyanobacteria and only four isolates successfully amplified a gene fragment from the myc E belonged to microcystin. Our results concluded that PCR assay can be used for early detection of microcystin producing algae in fresh water that useful to stations responsible for the preparation of drinking water.

Keywords: Algae, Cyanotoxin, phycocyanin and microcystin.

Introduction
Cyanobacteria, cyanophytes or blue green algae are widely distributed in natural environments and are considered a major component of microbial populations in terrestrial and aquatic habitats worldwide. Harmful algal blooms have been identified in fresh water, Estuarine, and marine system. In fresh water some cyanobacterial may produce dermal toxins, neurotoxins and hepatotoxins which including nodularins and microcystins (1).

They are also an interesting functional food source (2). These microbes have also been reported to be rich sources of healthy nutrients such as proteins, carbohydrates, vitamins, minerals amino acids, and fatty acids.

Among all the cyanotoxins, microcystins are the most frequently studied because of their wide distribution and high toxicity. Up to now, more than 80 different structural variants have been identified, among which microcystin-LR is the most common and potent variant, followed by microcystin-RR and microcystin-YR (3).

Monitoring systems are needed to prevent water users from these toxins. Good methods, such as ELISA and high performance liquid chromatography have been recorded for most cyanotoxins, but they extremely use laborious sample preparation protocols as well as priced machinery and purified toxin standards that are often difficult to obtain. Nevertheless, molecular detection techniques such as conventional PCR, quantitative real-time PCR and microarrays/DNA chips that are rapid, extremely sensitive and specific for detecting toxic cyanobacteria in water supplies (4; 5).

The delivery of phycocyanin in the cyanobacterial makes the study of phycocyanin genes good idea for the classification of cyanobacteria (6). Phycocyanin operon contains genes coding for two bilin subunits and three linker polypeptides. The intergenic spacer (IGS) between the two bilin subunit genes, designated as b (cpcB) and a (cpcA) showed variations in their sequences which are useful of differentiating genotypes below the generic level make it capable for the identification of cyanobacteria via PCR (7).
Conventional PCR could be useful to detect microcystin producers and several primers are available \(^{(8)}\). \(mcyE\) primers were successfully used to get PCR product from all known microcystin and nodularin producers \(^{(9)}\).

Baghdad city have two main sources of drinking water for Iraq. Tigris River is the most important once in serving population approximately seven million people settled in this city, this river usually affected by industrial eutification as well as the sewage effluent and agricultural which provide enhancement of cyanobacteria growth and potential mycrocystin production. Therefore, this study was aimed to use Molecular PCR technique for identification of cyanobacteria and mycrocystin producing isolates from Tigris River for early detection of toxic species that could be useful to companies responsible for the provider of drinking water to this city.

**Material and Method**

Water samples were collected from the higher superficial layer of Tigris River from a depth of 20-30 cm monthly in April 2017 by using 20μ mesh net. Sampling site located in civil region in AL-Great region near AL-Great bridge, located on longitude 44°20’37.52''E and latitude 33°25’3.49’’N. Samples were transported to the laboratory on ice. 10 ml of water sample was added to chu-10 culture medium and incubated at 28ºC for two weeks with continuous illumination of 50 μE/m²/s. one ml of growth inoculated on agar plates containing BG-11 and incubated in the same condition for one week to isolate unialgal \(^{(10)}\). Microscopic examination were performed to ensure the culture were unialgal.

Extraction of DNA from chlorophyta and cyanbacterial isolates

Genomic DNA was extracted from the chlorophyta isolates for specificity test using CTAB method \(^{(11)}\). While the Genomic DNA was extracted from the cyanobacterial isolates using the genomic DNA mini Kit (plant)

Polymerase chain reaction test

Polymerase chain reaction was performed with two sets of primers. PC\(\beta\)F (GGCTGCTTGTTTACGCGACA) and PC\(\alpha\)R (CCAGTACCACCAGCA ACTAA)\(^{(12)}\) to amplify cpc\(B\)-IGC-cpc\(A\) region in phycocyanin operon while the HEPF (TTTGGGTTAACTTTTTTGGG CATAGTC) and HEPR (AATTCTTGAGGCTTAATCGGTTT)\(^{(13)}\) used to amplify \(mcyE\) gene of the microcystin synthetase. PCR protocols involved an initial denaturation for 2 min at 95°C; 35 cycles of denaturation for 90 sec at 95°C, annealing for 30 sec at 52°C (PC\(\beta\)-PC\(\alpha\) primer set) and for 90 sec at 95°C (HEP primer set), extension for 1 min at 72°C and final extension for 8 min at 72°C. 10μl of PCR product was separate in 1.5% agarose gel electrophoresis stained with ethidium bromide and visualized on a UV transilluminator, the size of amplified products were compared with the 100pb DNA ladder to determine the exact size of these products.

**Results**

Isolation and identification of algae from water samples five isolates of cyanobacteria were obtained from the Tigris River included, Westellopsis sp , Oscillatoria sp , Spirulina sp , Chroococcus sp and Lyngbya sp. Which belonged to four cyanobacterial orders: Oscillatoriales, Chroococcales, Stigonematales and Nostocales as well as one isolate of chlorophyceae included Cladophora glomerata ( Macro algae ) where used as negative control test.

Molecular detection of cyanobacteria by PCR test

The gene fragment of the phycocyanin operon containing the IGS (cpcBA-IGC) from cyanobacteria was amplified. A distinct amplicon patterns was produced from all of the DNA extracts with a size of 650 bp when analyzed in gel electrophoresis (Fig. 1), confirming the presence of cyanobacterial DNA from isolates collected from fresh water of Tigris River in Baghdad. While lysates of a green alga Cladophora glomerata does not possess pycocyanin operon, gave no PCR product suggested the highly specificity of used primers.

Detection of Microcystin by PCR assay

In this study, conventional PCR used as a tool to identify potentially microcystin producing cyanobacteria possess aminotransferase enzyme. The HEP primers were successfully amplified the 472 bp fragments of \(mcyE\) gene from all microcystin-producing cyanobacterial isolates except spirulina sp. (Fig. 2). The specificity of HEP primers appeared to be highly specific for isolates producing microcystin since there was no DNA amplified from chlorophyta used in this study.
Fig. 1. Gel electrophoresis of amplified cpcBA-IGC (650bp) in cyanobacterial isolates. Agarose (1.5%), 5 V/cm for 2 hrs, stained with ethidium bromide and visualized on a UV transilluminator. M. 100 bp DNA ladder. Lane 1-5. Westellopsis sp, Oscillatoria sp, Spirulina sp, Chroococcus sp and Lyngbya sp. Lane C. Cladophora glomerata. M. Marker.

Fig. 2. Gel electrophoresis of amplified mycE (472bp) in cyanobacterial isolates. Agarose (1.5%), 5 V/cm for 2 hrs, stained with ethidium bromide and visualized on a UV transilluminator. M. 100 bp DNA ladder. Lane 1-5. Westellopsis sp, Oscillatoria sp, Spirulina sp, Chroococcus sp and Lyngbya sp. Lane 6. Cladophora glomerata. M. Marker.

Discussion

The microscopic results revealed that all species of cyanobacteria that isolated from Tigris River related to toxic dominant genera which produce microcystins except *Spirulina platensis*. This might be related to the capability of these species to highly competition to remain dominant utilizing all environmental conditions such as high temperature, optical density and abundance of nutrients, all these factors allowed to form the blooming and can increase microcystin production rates (14).

Morphological identification is time consuming and it requires high expertise. In fact, morphological features used for the identification of species such as
colonial form, mucilage patterns and cell arrangement in the colony is frequently variable and dependent on the environment (15). Furthermore, the co-occurrence of toxin producing and non-producing cells that are morphologically indistinguishable (16; 17). Therefore, the development of a molecular method for the identification of cyanobacteria is essential for the rapid and accurate analysis members of cyanobacterial population (6). 

Several investigators used PCβ-PCα primer set for cyanobacterial detection and showed the same results revealed in this study (12; 18). Except in (6) study, he was reported that phycocyanin gene fragments from Nostoc commune and Nostocpunctiforme were unable to be amplified using these primers while strains of all of the cyanobacterial genera were successfully amplified. In recent research, found that Nostoc punctiforme had short sequence and incomplete of cpcBA-IGC region resulting in high variability in these genes and cause heterogeneity of genus Nostoc(19).

The detection of cyclic peptide hepatotoxin genes by using HEPF and HEPR primers was developed to identify potentially microcystin or nodularin-producing cyanobacterial blooms that possess the aminotransferase domain of either mcy E or nda F, involved in the production of microcystin or nodularin from four order of cyanobacteria included Oscillatoriales, Chroococcales, Stigonematales and Nostocales (13).

The aminotransferase domain was chosen as the target sequence because of its essential function in the synthesis of all microcystins as well as nodularins that catalyzes the addition of D-glutamate to Adda, an essential step in the synthesis of both microcystin and nodularin (20). Thus, can use these described primers to amplify a 472 bp PCR product from the aminotransferase domains of all tested hepatotoxic species and bloom samples. In addition, these primers can be used for distinguished between toxic and non-toxic populations of cyanobacteria that coexist simultaneously in a single ecosystem and are indistinguishable by microscopy (13).

Tigris River usually affected by agricultural and industrial eutrification as well as the sewage effluent, high turbidity, river discharge or by agricultural runoff which provide protected mesocosms of cyanobacteria growth and potential microcystins production. The results by (21) and (22), suggested that eutrophication increased the co-occurrence of potentially microcystins producing cyanobacterial genera, raising the risk of toxic-bloom formation.

References


Flame Atomic Absorption Spectrophotometry Analysis of Heavy Metals in Some Food Additives Available in Baghdad Markets, Iraq

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Abstract

Flame atomic absorption spectrophotometer (FAAS) was used in this study to determine the concentrations of heavy metals such as Ca, Fe, Mn, Cd, Co, Cr, Ni, Cu, Pb and Zn in some food additives of Iraq. The order of metal contents in food additives was found to be Ca ˃ Mn ˃ Fe ˃ Cu ˃ Zn ˃ Pb ˃ Cr ˃ Ni ˃ Co ˃ Cd. The concentration level of each metal was compared with that recommended by food agriculture organisation (FAO) and world health organisation (WHO). Calibration curves were linear for all standard solutions of heavy metals in the range starting from 0.02-0.4 mg/kg for Cd to 11-100 mg/kg for Ca. The correlation coefficients values (R^2) of calibrations were investigated and ranged from 0.9971 for Cr to 0.9999 for Ca.

The limit of detection (LOD) and limit of quantification (LOQ) were found to be in highest value for Ca (1.6569 mg/kg and 5.5232 mg/kg), while they were found to be in lowest values for Cd (0.0150 mg/kg and 0.0499 mg/kg).

Keywords: Heavy metals, Food additives, Flame atomic absorption spectrophotometer

Introduction

Several elements are important for a wide range of biological processes due to their conjugation with proteins to form metalloproteins which are an important component in the enzymatic systems. Despite the importance of some metals including Cr, Al and Ni for plants, they can be toxic with high concentrations. Furthermore, some heavy metals such as Pb and As in food are a major health problem due to their carcinogenic effects. In addition, the presence of metals in soils during wastewater are environmental problem, and the main cause of the agricultural pollution.

The last three decades have seen increasing the production of spices about 3.5% per year, and play a key role in the history of civilisation around the world. Although spices are essential, there is increasing concern that some of them are being disadvantaged. This attributed to the accumulation of toxic trace elements.

In addition, the spices may be contaminated with trace and heavy metals, and this could lead to health problems. Therefore, researches have shown an increased interest in examine the effect of metals on air, water and food and their impact on human consumption. Concentrations of trace and heavy metals in spices are an important components in the healthcare systems due to their medicinal effective on human health. Some metals such as lead (Pb), arsenic (As), nickel (Ni) and cadmium (Cd) can be extremely harmful to human beings even in trace levels. However, spices are one of the most widely used groups of antibacterial, antioxidant and anti-diabetic agents.

A number of studies have been made to investigate the concentration of metals in spices. On the other hand, information about the efficacy of heavy metals and safety of spices are limited. The heavy metals in food need to be monitored in order to protect human and animals from the hazards of these metal ions; WHO has determined the maximum level for each toxic element in foodstuffs. Several analytical methods have been used to determine the metal concentrations in spice samples.
different countries. Krejpcio and co-workers have shown the quantification analysis of some heavy metals such as Cd, Pb, Cu and Zn in popular spices used in Polish market using atomic absorption spectrophotometer (AAS)\(^{11}\). In the study by Nkansah and Amoako, AAS has been successfully used to monitor the concentration levels of heavy metals including Ni, Fe, Cu, Co, Pb and Zn in many different types of spices in Ghana\(^{9}\). The quantification analysis of some toxic metals such as As, Pb, Cd and Ni in a number of common spices in Pakistan using AAS method were carried out by Baig \textit{et al}\(^{8}\). In a study conducted by Karadas and Kara, it was shown that geometric methods such as principal component analysis (PCA) and cluster analysis (CA) can be used to analyse the trace metal concentrations such as Co, Mn, Fe, Mg, Cr, Cu, Ca, Cd, Ba, As and Sr in some spices in Turkey\(^{15}\).

The experimental work presented here provides the levels of heavy metals in some food additive available in the local markets of Baghdad, Iraq. This study therefore set out to assess the effect of some heavy metals such as Ca, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in some popular food additive in Iraq such as qayima, dolma, kubba, seven, pizza, biryani, judur, chicken, kubsa, burger and sumac using FAAS technique. Both qualitative and quantitative methods were used in this investigation.

**Materials and Method**

**Apparatus**

Flam atomic absorption spectroscopy (FAAS) model AA-7000 Shimadzu was used as instrumental detection system using hollow cathode lamps. Absorbance measurements of blank (solvent) were recorded using deuterium lamp. Air/ acetylene burner head was used as a carrier gas for all samples. The wavelength used for each metal was: Ca (422.7 nm), Fe (248.3 nm), Mn (279.5 nm), Cd (228.8 nm), Co (240.7 nm), Cr (357.9 nm), Ni (232 nm), Cu (324.8 nm), Pb (283.3 nm) and Zn (213.9 nm).

**Chemicals**

All chemicals were used without any further purification and all solutions were prepared by deionised water. Nitric acid (65 %) was supplied by Riedel-de Haen. Standard solution of each metal that used for the calibration curve was prepared by stock solution (1000 mg/L) supplied by Aldrich. Deionised water was provided by Daihan Labtech CO., LTD (Model WD-2008F) with a volts of 220V 50 Hz.

**Preparation of samples**

Eleven samples of food additives such as qayima, dolma, kubba, seven, pizza, biryani, judur, chicken, kubsa, burger and sumac that used in the study were collected by the local markets of Baghdad, Iraq. One gram of each sample was weighed and digested by mixing with 2 ml of concentrated nitric acid and heated for 1 hour. Then, the produced samples were cooled at room temperature. After digestion, the samples were filtered and diluted to 25 ml with deionised water.

**Analysis of sample**

All prepared sample solutions were analysed by FAAS method to determine the concentrations of heavy metals such as Ca, Fe, Mn, Cd, Co, Cr, Ni, Cu, Pb and Zn that used in this study. Blank solution was also measured before the sample analysis using the same conditions.

**Quantification**

For quantification, calibration curves of five different concentrations of each standard solution were applied. All calibrations curves showed a good linear correlation between the concentrations of standard solutions and absorbance. Some statistical analysis including the standard deviation (S.D.), standard error of the mean (S.E.M.) and correlation of coefficients (R\(^2\)) were done in order to check the validation of FAAS method. All R\(^2\) were found to be ≥ 0.997. The limit of detection (LOD) and the limit of quantification (LOQ) were also determined by considering standard deviation to the slope:

\[
\text{STEYX means the standard deviation of the y-value and x-value.}
\]

**Results and Discussion**

FAAS has been used to determine many heavy metals such as Ca, Fe, Mn, Cd, Co, Cr, Ni, Cu, Pb and Zn in some food additives such as qayima, dolma, kubba, seven, pizza, biryani, judur, chicken, kubsa, burger and sumac. The results obtained from the analysis are presented in Table 1. The samples were prepared in the same solvent used for the analysis. The wavelength was set for each element (see the material and method section). It can be seen from the date in Table 1 that the level of Ca in all samples is higher than levels observed.
of other metals. Interestingly, the samples of qayima and judur did not detect any value of Cr and Co. The results of the study are in agreement with those obtained by WHO. Therefore, there was no evidence that the levels of heavy metals concentrations in these food additives has an influence on human consumption.

Table 1: Contents of heavy metals in different food additives in mg/kg

<table>
<thead>
<tr>
<th>Heavy metals</th>
<th>Ca</th>
<th>Fe</th>
<th>Mn</th>
<th>Cd</th>
<th>Co</th>
<th>Cr</th>
<th>Ni</th>
<th>Cu</th>
<th>Pb</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qayima</td>
<td>45.840</td>
<td>6.657</td>
<td>6.519</td>
<td>0.009</td>
<td>0.018</td>
<td>0.000</td>
<td>0.028</td>
<td>0.291</td>
<td>0.024</td>
<td>1.106</td>
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<tr>
<td>Dolma</td>
<td>48.146</td>
<td>5.785</td>
<td>7.146</td>
<td>0.005</td>
<td>0.018</td>
<td>0.030</td>
<td>0.028</td>
<td>2.148</td>
<td>0.048</td>
<td>1.056</td>
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<td>Kubba</td>
<td>40.089</td>
<td>3.133</td>
<td>6.188</td>
<td>0.007</td>
<td>0.016</td>
<td>0.008</td>
<td>0.024</td>
<td>1.442</td>
<td>0.072</td>
<td>0.836</td>
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<td>Seven</td>
<td>47.985</td>
<td>6.184</td>
<td>6.362</td>
<td>0.005</td>
<td>0.015</td>
<td>0.015</td>
<td>0.046</td>
<td>3.047</td>
<td>0.167</td>
<td>1.182</td>
</tr>
<tr>
<td>Pizza</td>
<td>47.725</td>
<td>5.134</td>
<td>6.559</td>
<td>0.015</td>
<td>0.034</td>
<td>0.060</td>
<td>0.055</td>
<td>0.402</td>
<td>0.127</td>
<td>0.842</td>
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<td>Biryani</td>
<td>62.550</td>
<td>5.889</td>
<td>7.611</td>
<td>0.023</td>
<td>0.015</td>
<td>0.067</td>
<td>0.041</td>
<td>0.334</td>
<td>0.270</td>
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<td>Judur</td>
<td>37.324</td>
<td>6.683</td>
<td>4.710</td>
<td>0.012</td>
<td>0.000</td>
<td>0.104</td>
<td>0.009</td>
<td>0.273</td>
<td>0.040</td>
<td>0.928</td>
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<tr>
<td>Chicken</td>
<td>45.555</td>
<td>6.562</td>
<td>5.930</td>
<td>0.006</td>
<td>0.023</td>
<td>0.052</td>
<td>0.047</td>
<td>2.414</td>
<td>0.095</td>
<td>0.970</td>
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<tr>
<td>Kubsa</td>
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<td>5.888</td>
<td>0.005</td>
<td>0.030</td>
<td>0.074</td>
<td>0.043</td>
<td>1.101</td>
<td>0.048</td>
<td>0.993</td>
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<td>Burger</td>
<td>69.058</td>
<td>6.258</td>
<td>4.843</td>
<td>0.008</td>
<td>0.033</td>
<td>0.037</td>
<td>0.110</td>
<td>0.855</td>
<td>0.087</td>
<td>0.575</td>
</tr>
<tr>
<td>Sumac</td>
<td>7.896</td>
<td>3.329</td>
<td>1.319</td>
<td>0.016</td>
<td>0.018</td>
<td>0.134</td>
<td>0.129</td>
<td>1.668</td>
<td>0.024</td>
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</tbody>
</table>

The results obtained from the Figure 1, it can see that the burger recorded the highest level of Ca (69.058 mg/kg), whereas the lowest level was found in sumac (7.896 mg/kg). From the data in Figure 1, it can see that the study resulted the concentration levels of Fe in the samples and found to be between 3.133 mg/kg in kubba and 6.683 mg/kg in judur. These values are lowest than that of determined by FAO and WHO, 2009 (20 mg/kg). Compared to permissible limit of FAO and WHO, 1984 (2 mg/kg), there was the higher Mn content in all food additives samples (> 4 mg/kg) except sumac, Figure 1.

Figure 1: Levels of Ca, Fe and Mn in different food additives
As can be seen from the Figure 2, the concentrations of cadmium in all food additives were lower than that determined by FAO and WHO, 1984 (0.2 mg/kg). They found to be in the range from 0.005 mg/kg in dolma to 0.023 mg/kg in biryani. As shown in Figure 2, the concentrations of “Co” in all samples were under the permissible limit of FAO and WHO, 1984 (0.4 mg/kg). They were in the range of 0.015- 0.034 mg/kg in seven, biryani and pizzaa, whereas judur sample did not show any content of Co. Only trace amounts of Cr were detected in the food additives samples, however, FAO/WHO (2009) suggested that the concentration of Cr should be zero in these samples. When Cr was found from 0.008 mg/kg in kubaa to 0.134 mg/kg in sumac, no amount in qayima was detected. The results also shows that the greatest value of Ni was in sumac (0.129 mg/kg), while the lowest value was in judur (0.009 mg/kg). This data can be compared with that determined by FAO and WHO, 1984 (1.63 mg/kg). As shown the maximum obtained values were too far from the permissible limit of FAO and WHO, therefore, the selective food additives in this study appeared to be unaffected by these levels of Ni.

It can be seen from the Figure 3 that the food additives samples reported significantly high concentrations of copper compared to those of some heavy metals such as Cd, Co, Cr, Ni and Pb. It found to be between 0.273 mg/kg in judur and 3.047 mg/kg in seven. However, these observed concentrations were in general under the maximum limit of FAO and WHO, 1984 (3.00 mg/kg). From the data in this Figure, it is apparent that the Pb levels in food additives were higher than those of Cd and Co and lower than other metals. The range of concentrations were found from 0.024 mg/kg in both qayima and sumac to 0.270 mg/kg in biryani. This results indicated there was no evidence that Pb has an influence on human health due to their levels were under the permissible limit of FAO and WHO, 1984 (5.00 mg/kg). The results also shows that the levels of Zn ranged from 0.575 mg/kg in burger to 1.360 mg/kg in biryani. Therefore, they were too far from the maximum level that recommended by FAO and WHO, 2009 (50 mg/kg).

Correlation analysis of five different concentrations including the blank of each metal were tested to predict the linearity. Calibration ranges of standard solutions are set out in Table 2. From the Figure 4, it can see that all calibrations showed a good linearity over the range of concentrations. The LOD and LOQ of Ca is expected to be higher than others, whereas for Cd they were found to be lower than others. Other values of LOD and LOQ were listed in Table 2. Standard deviation (S.D.) and standard error of the mean (S.E.M.) were calculated by the equations:

\[ S = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n - 1}} \]

\[ \text{S.E.M.} = \frac{s}{\sqrt{n}} \]

Where \( S \) is standard deviation, \( n \) is number of values in the sample, \( x_i \) is data value, \( \bar{x} \) sample mean, S.E.M. is standard error of the mean and \( s \) is size of the sample. The results of the mean, S.D. and S.E.M. are summarised in Table 2.
**Table 2: Data of linear regression, sensitivity and precision of the FAAS method of the FAAS method**

<table>
<thead>
<tr>
<th>Metal</th>
<th>Calibration Range (mg/kg)</th>
<th>Corr. R²</th>
<th>LOD (mg/kg)</th>
<th>LOQ (mg/kg)</th>
<th>S.D.</th>
<th>S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>11-100</td>
<td>0.9999</td>
<td>1.6569</td>
<td>5.5232</td>
<td>15.7482</td>
<td>4.7482</td>
</tr>
<tr>
<td>Fe</td>
<td>0.5-10</td>
<td>0.9992</td>
<td>0.3813</td>
<td>1.2825</td>
<td>1.2603</td>
<td>0.3800</td>
</tr>
<tr>
<td>Mn</td>
<td>0.4-7.0</td>
<td>0.9991</td>
<td>0.2975</td>
<td>0.9959</td>
<td>1.6972</td>
<td>0.5117</td>
</tr>
<tr>
<td>Cd</td>
<td>0.02-0.4</td>
<td>0.9993</td>
<td>0.0150</td>
<td>0.0499</td>
<td>0.0057</td>
<td>0.0017</td>
</tr>
<tr>
<td>Co</td>
<td>0.4-4.0</td>
<td>0.9991</td>
<td>0.1696</td>
<td>0.5654</td>
<td>0.0096</td>
<td>0.0029</td>
</tr>
<tr>
<td>Cr</td>
<td>0.1-2.0</td>
<td>0.9971</td>
<td>0.1505</td>
<td>0.5019</td>
<td>0.0412</td>
<td>0.0124</td>
</tr>
<tr>
<td>Ni</td>
<td>0.1-2.0</td>
<td>0.9997</td>
<td>0.0518</td>
<td>0.1727</td>
<td>0.0372</td>
<td>0.0112</td>
</tr>
<tr>
<td>Cu</td>
<td>0.3-4.0</td>
<td>0.9987</td>
<td>0.1974</td>
<td>0.6581</td>
<td>0.9595</td>
<td>0.2894</td>
</tr>
<tr>
<td>Pb</td>
<td>2.5-10</td>
<td>0.9988</td>
<td>0.4823</td>
<td>1.5951</td>
<td>0.0741</td>
<td>0.0223</td>
</tr>
<tr>
<td>Zn</td>
<td>0.1-1.0</td>
<td>0.9997</td>
<td>0.0209</td>
<td>0.0698</td>
<td>0.0614</td>
<td>0.0238</td>
</tr>
</tbody>
</table>

**Figure 4. Calibration curves for the standard solutions of heavy metals by FAAS**

**CONCLUSION**

The present study was designed to determine the levels of some heavy metals in different samples of food additives available at local markets in Baghdad, Iraq using FAAS technique. This has shown that the method is simple, fast and sensitive. The obtained concentrations of metals were acceptable with that recommended by FAO and WHO. The study has shown that highest correlation coefficient (R²) of calibrations was found to be for Ca (0.9999), while the lowest was found to be for Cr (0.9971). The LOD and LOQ for Cd were found to be lower than others (0.0150 mg/kg and 0.0499 mg/kg) followed by Zn (0.0209 mg/kg and 0.0698 mg/kg). This could attributed to that the Cd and Zn are sensitive enough even at low concentrations.

For recommendations, the findings of this study have several important applications for future work. The method can be applied for determination of other metals in different samples of foodstuffs. Further research should also focus on determining the toxic and carcinogenic heavy metals in food additives and how can be able to reduce their effects by reducing their amounts.
Acknowledgment: The authors thank the Central Service Laboratory at College of Education for Pure Science (Ibn Al- Haitham), University of Baghdad for providing the facilities to carry out this work. The authors would also like to thank Department of Chemistry for providing consultancies.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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References

Detection Malta Fever by Interferon-gamma and Steroid Hormone S Level

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Abstract

Malta fever is one of the most common bacterial zoonosis, its causes abortion of pregnant women. Abortion is the chief obvious manifestation of Brucella infection. Brucella like better cattle placenta as a result of great concentration of erythritol sugar, whereas human placenta there is no erythritol sugar only steroid hormone, for this reason designed the our project . In this study, 100 aborted women were included , where referred to maternity and children hospital of Babylon province \ Iraq. Diagnosis of Brucella infection in these abortions was concentrated on serological and bacteriological technique. Serological studies included the use of RB and ELISA tests. Aggressive differences between RB and ELISA results have been shown. Brucella isolated and identified from aborted placenta and blood samples were 7 (7%) isolates from aborted women.

Hormonal assessment by Immunohistochemical technique in Brucella infected women, showed significant decrease in progesterone expression in comparison with that aborted due to other causes, in other hand Brucella infected women showed high expression in estrogen hormones . ELISA technique was the valuable serological test to confirm the diagnosis of brucellosis as compared with RB test.

Keyword: Malta fever , Brucella , Interferon-gamma , Steroid hormone

Introduction

Malta fever also called Brucellosis, is a zoonotic disease effecting humans and animals in many countries e.g, Mediterranean area, Iraq, middle east, India, and America(1). Reports from the regions where Brucella melitensis infection is endemic, propose that there is an increased rate of abortion in asymptomatic pregnant women. The diagnostic method acknowledged to produce the best results in terms of specificity is the isolation of Brucella organisms from the suspected human , Different Brucella species are recognized as causative agents of brucellosis and some of them are acknowledged to be pathogenic to humans (2-8). However, Because of the variety of the disease and its non-specific clinical manifestation, the clinical diagnosis of Malta fever remains a challenge. Malta fever (Brucellosis) mimics other infectious and non-infectious diseases, resulting in a delay in diagnosis or misidentification of the disease(9). The diagnosis is importantly dependent on a epidemiological history , clinical signs , patient’s medical, biochemical testing , hematological, radiological examination and, necessarily, on Brucella-specific laboratory tests. Very important sides for correct and fast diagnosis are the disease-specific laboratory tests and knowledge of their weaknesses, proper analysis and correct assessment of their results (10). Rose Bengal (RB) is the main serological test used to identify antibodies against brucellosis. Because of the difficulties in the method and low sensitivity of the isolation methods, laboratory diagnosis relies largely on serological tests. The major antigens of Brucella being used in serological testing are the internal-cytosolic proteins and lipopolysaccharide (smooth-S LPS) (11). Brucella Lipopolysaccharide is a strong immunogen but its epitopes are the chief etiology of cross-reactions with other Gram-negative bacteria , a condition that creates evaluation more difficult(12). Cytokines have an important role in the pathogenesis of Malta fever and the Th1/Th2 balance may include in the resistance or susceptibility to the disease (13).
are responsible for control of the infection. Experimental studies showed that IFN-γ is essential for exclusion of Brucella and for host survival in case of virulent Brucella challenge (14). The aims of the present work were:

1- Trophism of Brucella to uterus of women that it not has erythritol sugar

2- Evaluate steroid hormones level in placenta and blood is specific method to detection of Malta fever.

3- Evaluate interferon-gamma level in blood.

Materials and Methods:

Human brucellosis kit was provided by Elabscience/China. Gram’s stain solution. Rose Bengal antigen was provided by Omega company, UK. antibrucella abortus, antibrucella melitensis and Monospeciic antiserum were supplied by Difco, USA.

Methods

Samples collection.

1. Clinical signs of aborted women were recorded by physician to show pregnancy period and sings of abortion.

2. Blood samples: Hundred blood samples from women were obtained. serum samples have been obtained for serological and immunological assessments using RB and ELISA tests.

3. Aborted Placentas:

   Hundred placenta samples from aborted women have been obtained, at maternity and children hospital of Babylon province, placenta samples were cultured directly on Brucella selective medium and blood agar.

4. Immunohistochemistry for placenta specimens was performed as described by (Dako, UK). The expression of “estrogen and progesterone hormones were measured as the same scoring system” used by Mao et al., (15). The positivity of cells for expression of hormones were seen as brown staining. It was graded as four grad of the cells staining positive for hormones.

<table>
<thead>
<tr>
<th>Score</th>
<th>0</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
<th>4+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Cells</td>
<td>&lt;10%</td>
<td>10-25%</td>
<td>25-50%</td>
<td>50-75%</td>
<td>&gt;75%</td>
</tr>
</tbody>
</table>

5- IFN-gamma ELISA performed as described by (RayBiotech. Inc).

Statistical Analysis:

The results are expressed at percentage by Chi-square test, (SPSS) for Windows program was used to compare between the frequencies. Student t test was used to compare between means of groups. The significance was accepted as P value < 0.05.

Results

Clinical signs in women included fever and bleeding, most women infected with Brucella were aborted at first stage of pregnancy.

Serological tests:

ELISA, RB test revealed that RB results were positive in 27 cases (27%). ELISA results were positive in 14 cases(14%) . according to Pearson Chi-Square test, the difference in RB and ELISA positive cases was significant (P<0.05) show in Table (1).

Table (1) showed the diagnostic test of Brucella infected women

<table>
<thead>
<tr>
<th>Blood samples</th>
<th>RB</th>
<th>%</th>
<th>Culture(+)</th>
<th>%</th>
<th>ELISA</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>27</td>
<td>27</td>
<td>7</td>
<td>7</td>
<td>14</td>
<td>14</td>
</tr>
</tbody>
</table>

Bacterial isolation & identification:

Out of the 100 aborted women, 7 (7%) were positive for culture, from 14 patient blood samples that positive for positive ELISA, 7 isolates were positive by culture, after 2-4 days the Brucella culture recognized on the basis of colonial morphology (translucent , round with pearly appearance).

Isolates from blood and placenta samples were Gram-negative, coccobacilli, arranged singly in short chain or small groups stained with modified ziehl-
neelsen stain , Biochemical test of Brucella show in table (2)

Table (2): Biochemical test of Brucella

<table>
<thead>
<tr>
<th>Test</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Haemolysis</td>
<td>-</td>
</tr>
<tr>
<td>2 macconkey agar</td>
<td>-</td>
</tr>
<tr>
<td>3 indol</td>
<td>-</td>
</tr>
<tr>
<td>4 MR-VP</td>
<td>-</td>
</tr>
<tr>
<td>5 gelatinase</td>
<td>-</td>
</tr>
<tr>
<td>6 Citrate utilization</td>
<td>-</td>
</tr>
<tr>
<td>7 urease</td>
<td>+</td>
</tr>
<tr>
<td>8 nitrate reduction</td>
<td>+</td>
</tr>
<tr>
<td>9 catalase</td>
<td>+</td>
</tr>
</tbody>
</table>

Steroid hormones assessment by immunohistochemistry assay:

Result of imunnohistochemical assay showed positive staining for progesterone hormone in placenta of aborted woman non infected with Brucella , compared with that of positive for Brucella infection which exposed low intensity for IHC staining of progesterone, the score differences were also seen in (table 3).

Table (3) : Existence of progesterone molecule in placenta of aborted women,(IHC assay)

<table>
<thead>
<tr>
<th>Score</th>
<th>Positive for Brucella infection</th>
<th>Negative for Brucella Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO</td>
<td>%</td>
<td>NO</td>
</tr>
<tr>
<td>1</td>
<td>3 42.58</td>
<td>0 0</td>
</tr>
<tr>
<td>2</td>
<td>4 57.14</td>
<td>1 14.28</td>
</tr>
<tr>
<td>Total of negative score</td>
<td>*100%</td>
<td>14.28%</td>
</tr>
<tr>
<td>4</td>
<td>0 0</td>
<td>1 14.28</td>
</tr>
<tr>
<td>5</td>
<td>0 0</td>
<td>5 71.42</td>
</tr>
<tr>
<td>Total of positive score</td>
<td>0%</td>
<td>85.71%</td>
</tr>
</tbody>
</table>

*Significant (p≤ 0.05)

According to study the estrogen particles , there is obvious rise for estrogen stain for placenta tissue through Brucella infection , as determined by staining of biopsies , the immune staining of estrogen were positive at high level in 85.71% (6 out of 7) in Brucella infected patients, with highly statistical association (p≤ 0.05) between the infected & non infected groups (table 4).
Interferon-gamma assessment by ELISA technique

Patients with Malta fever had significantly (P<0.05) higher serum levels of IFN-γ (175.078 ± 69.821 pg/ml) compared to control group (39.358 ± 29.847 pg/ml) shown in (Figure 1).

![Figure 1: Serum concentration of IFN-γ in patients and control](image)

Table (4) : Amount of estrogen molecules in placenta of aborted women (IHC assay)

<table>
<thead>
<tr>
<th>Score</th>
<th>Negative for Brucella infection</th>
<th>Positive for Brucella Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO</td>
<td>%</td>
<td>NO</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

Score : 1< 25% ; 2 (25-74)% ; (75-100)%

Discussion

RB gave positive result in aborted women but were negative for ELISA and bacterial isolation infection, because the RB test is rapid and screening test and may be give cross-reaction with other Gram negative bacteria example : E.coli O:157, Yersinia enterocolitica O:9, Vibrio cholerae O:1, Salmonella spp., Francisella tularensis and Pseudomonas maltophilia, and give false positive for RB (16).

Antibodies to Brucella appear in the serum within (1-2) weeks of infection. The initial response is the appearance of IgM isotype (which can be easily detected by RB) followed by a switch to IgG, after a while titers of both Immunoglobulins classes increase distinct most of the usual serological tests, ELISA is effective in distinguishing all immunoglobeline (antibodies) classes and sub-classes essential in diagnosis and appears to be the most sensitive serological test increase in IgG but not IgM. IgA titres roughly paralleled IgG titers (17). ELISA using S-LPS Ag can be used to measure the development of immunoglobulin isotypes following infection and after treatment (18). Steroid hormones concentration in aborted women positive for brucellosis were showed decrease in progesterone levels and increase in other hormones due to Brucella infection, aborted women negative for brucellosis notice significant increase in hormone , this certified that other cause of abortion may be not effect on progesterone synthesis.

Estimation of hormones are more effective method to conformation of Brucella infection, while other serological methods may be causes cross-reaction and false positive results.

There are two HSD3B1 proteins, labeled type I and type2 that are expressed by different genes and function in different regions of the body (19). HSD3B1 has too been shown to be there a highly specific and sensitive trophoblast-associated marker, also showed that expression of 3β-HSD in trophoblast more than 50% considered as positive cells for 3βHSD (20). To form steroid hormones, the subsequent processing of pregnenolone requires enzymes related to smooth endoplasmic reticulum, such as 17-α-hydroxylase (P450c17α) and 3β-hydroxysteroid dehydrogenase/isomerase (3βHSD) pregnenolone is converted to progesterone by the enzyme 3β-HSD, which in turn, is converted to androstenedione by the enzyme 17α-hydroxylase/C17, 20 – lyase (P450c17α) (21).

In this study, have high serum levels among patients with Malta fever compared with control. These results are in agreement with earlier studies (22) who reported height this cytokine in Malta fever patients.
Most studies specified that CD4+ T lymphocytes are the main producer of IFN-γ although other subsets such as “CD8+ T lymphocytes, γδ T lymphocytes and NK cells also participate in the production of this cytokine (23). As there are relatively high serum levels of IFN-γ, it indicates an enlarged number of CD4+ which, in turn, indicates a chronic infection” (24).

**Conclusion**

In conclusion, placental immunohistochemical and assessments for steroid hormones (progesterone) and interferon-gamma have an efficient diagnostic values which can be included for confirmation of brucellosis.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** Self-funding

**References**


Hepatic Toxicity in Patients with Rheumatoid Arthritis and Psoriasis Taking Methotrexate Therapy

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Abstract

Background: We had made a study to demonstrate the adverse hepatic effects of MTX in patients taking MTX for treatment of RA and psoriasis taking in consideration the following variables: BMI, gender, cumulative dose, age, weekly dose, duration of treatment, serum level of cholesterol and creatinine.

Patient and method: We had a prospective study of 85 patients with RA and 50 patients with psoriasis. All patients were analyzed by history, clinical examination and investigations in the form of liver enzymes, blood sugar, serum cholesterol, serum creatinine, HBS Ag and anti HCV antibody. Persistently elevated level of liver enzymes 2 to 3 times the upper limit of normal on two occasions 3 months apart indicate hepatic toxicity.

Results: We found that 7 patients with psoriasis and 6 patients with RA have significant elevated liver enzymes which reflect MTX hepatotoxicity.

Conclusion: Our study show that patients with psoriasis at significantly greater risk of elevated liver enzymes than patient with RA (14% and 7% respectively) were gender, BMI, cumulative dose, weekly dose and serum cholesterol level are risk factors for hepatic toxicity due to MTX therapy.

Key words: Hepatic Toxicity, MTX therapy, Arthritis

Introduction

Methotrexate is a folic acid antagonist that inhibits dihydrofolate reductase. DNA synthesis is inhibited as the concentration of thymidine and purines falls after treatment with methotrexate. The relevant targets of low dose methotrexate have not been defined with precision, but an attractive candidate is the enzyme 5- amino-imidazole -4- carboxamide ribonucleotide (AICAR) transformylase. Inhibition of AICAR transformylase leads to accumulation of AICAR, which in turn stimulate the extracellular release adenosine which has a number of anti-inflammatory and immunomodulatory effects that may contribute to the therapeutic effect of methotrexate. Recently, in vitro studies showed that methotrexate was 10-100 times more effective at inhibiting the proliferation of lymphoid cell lines than cultured keratinoocytes, suggesting that lymphoid cells may be a more important cellular target than epithelial cells in psoriasis and also inhibits polymorpho nuclear leukocyte chemotaxis. These actions may explain its clinical effect.

Methotrexate is now the most widely used disease modifying antirheumatoid drug (DMARD) in the developed world. It was first used in the treatment of psoriasis and psoriatic arthritis in 1951, and has been shown to be of clinical benefit in this condition.

Methotrexate has been used for the treatment of diseases characterized by inflammation or cellular proliferation. In 1985, the first randomized placebo-controlled trials were published that demonstrate the short term efficacy of low dose weekly methotrexate in Rheumatoid arthritis.

Comparative studies of methotrexate with azathioprine, gold sodium thiomalate and cicolospirone in the treatment of Rheumatoid arthritis show that methotrexate is well tolerated, with retention rates between 93% and 95%.

Oral therapy is given once a week. Daily dose schedules are dangerous and have been abandoned. The oral triple-dose regimen is the most common method
used. A dose is taken at 12 hours intervals during a 36 hours period once each week. An initial test dose of 2.5 to 5mg is given and complete blood cell counts and liver function tests are obtained once week later. Maintenance doses should be achieved by gradual increases of 2.5-5mg per week up to 20mg/week (12).

The most common adverse effects are gastrointestinal, such as anorexia, nausea, vomiting, stomatitis and diarrhoea. Central nervous system toxicity including headache, dizziness, fatigue and mood disturbance may occur. Haematological toxicity is not common, and although all types of cytopenia has been documented. Pulmonary complications such as methotrexate pneumonitis may be linked to risk factors as increased age, diabetes mellitus and pre-existing pulmonary diseases (13). It is now thought that the frequency of severe liver disease occurring in rheumatoid arthritis patients receiving long term methotrexate is not high; the incidence of mild fibrosis has been reported as being between 0% and 2% (14-15).

Base line monitoring include history, physical examination, complete blood count, platelet count, renal function test, liver chemistry including (AST, ALT, alkaline phosphate, albumin) and viral serology mainly for hepatitis B, C and HIV antibody and chest radiography to exclude pulmonary fibrosis. Follow up monitoring include monthly complete blood count and differential, platelet count for the first and second dose then every two months, liver chemistries monthly or every two months, and renal function test including blood urea, serum creatinine at three to four months interval.

Persistently elevated liver enzymes more than two times would preclude further therapy. Although combined sensitivity of liver enzymes for detecting a significantly abnormal liver biopsy is 86%, whereas the predictive value of negative test result 93% (16), liver biopsy remain more reliable predicator of liver damage. Current study aimed to Assess the prevalence of liver enzyme abnormality in patients with RA and psoriasis taking MTX therapy and to identify the possible risk factors for MTX induce hepatotoxicity in these patients.

Patients and Method

Eighty five patients with rheumatoid arthritis diagnosed according to American college of rheumatology criteria (17) and 50 patients with psoriasis diagnosed by the presence of psoriasis with or without seronegative peripheral arthritis (18), underwent a prospective study

The study was performed in Marjan Teaching Hospital in Babylon for outpatient clinic of rheumatological and dermatological disease. After taking the verbal consents of the patients, full history regarding age, gender, MTX dose per week, MTX duration, and cumulative dose defined as dose per week multiplied by duration of treatment, MTX adverse effects mainly the gastrointestinal problems (nausea, vomiting, abdominal pain and anorexia). Oral MTX therapy and folic acid supplement prescribed for all patients (5mg once daily). Drug history concentrated on (NSAIDs, statins, cordaron, oral hypoglycemic drugs, psoraline+UVA treatment, gold, oral contraceptive pills, long term steroid and extreme obesity), any patient on these drugs for the last month were excluded, history of alcoholism so any patient drunk alcohol in the last 5 years were excluded from the study.

History of other comorbid disease like congestive heart failure, chronic viral hepatitis, autoimmune hepatitis, Wilson’s disease, chronic renal failure and diabetes mellitus were excluded from the study. Clinical examination include jaundice, ascites, organomegaly was performed, BMI represent the height and weight recorded as Wt. in Kg / Height in m² were 18 - 25 considered normal, 25 - 29.9 considered overweight, 30 – 39.9 considered obese and >40 considered extreme obesity (19). Investigations in the form of liver enzymes (normal reference values for ALT, AST <20 U/100 ml, normal reference for ALP, 85 U/100 ml) done by colorimetric method, random blood sugar normal reference value <11.1 mmol/L by glucose oxidase method, cholesterol level normal reference value <5.2 mmol/L by cholesterol oxidase method, serum creatinine normal reference value <124µmol/L by alkaline picate with Deprot method and viral serology for HBS Ag, anti HCV Ab. By bioelisa color method (direct immune enzymatic method). Persistently elevated level of liver enzymes 2 – 3 times the upper limit of normal on two occasions 3 months apart is indicate hepatic toxicity (20).

Statistical Analysis

Statistical significance of liver enzymes between patients with RA and psoriasis, and each risk factors were assessed by t-test and of the proportion, were p-value < 0.05 indicate Statistical significance the results were expressed as tables.
Results

The average age of patients with RA was 48 years and for patients with psoriasis was 50 years, female outnumbered male in RA group were 56 to 29 and for psoriasis group, the female outnumbered male 23 to 27. Body mass index in RA group was 24 kg/m² and for psoriasis group was 27 kg/m². The average duration of MTX therapy was 4 years in RA group and 3 years in psoriasis group. The average dose of methotrexate therapy in RA group was 10 mg/week and for psoriasis group was 15 mg/week [Table 1].

Regarding gastrointestinal symptom as nausea and vomiting were found in 20 patients (23.5%) with RA and in 10 patients (20%) with psoriasis [Table 2], statistically not significant.

Sustained rise in liver enzyme were seen in 6 patients (7%) in RA group while in 7 patients (14%) in psoriasis group [Table 3] which was significantly significant. The gender, liver enzyme abnormalities were found in 2 males (33.3%) and in 4 females (66.6%) in RA group while 4 males (57.1%) and in 3 females (42.8%) in psoriasis group [Table 4], statistically not significant. The age, the average age in RA was 46 and 52 years in psoriasis group [Table 5], statistically not significant. BMI, in RA group was 23 kg/m² and in psoriasis group was 28 kg/m² [Table 6], statistically significant.

**Table 1) Demographics of study population**

<table>
<thead>
<tr>
<th>Variable</th>
<th>RA</th>
<th>Psoriasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of patients</td>
<td>85</td>
<td>50</td>
</tr>
<tr>
<td>Average age (year)</td>
<td>48(15-65) ± 12</td>
<td>50(18-65) ±13</td>
</tr>
<tr>
<td>Female</td>
<td>56(65%)</td>
<td>23(46%)</td>
</tr>
<tr>
<td>Male</td>
<td>29(35%)</td>
<td>27(44%)</td>
</tr>
<tr>
<td>Average of BMI Kg/m²</td>
<td>24(17-30) ±2</td>
<td>27(20-35) ±4</td>
</tr>
<tr>
<td>Average dose of MTX Mg/week</td>
<td>10(5-10) ±3</td>
<td>15(10-20) ±4</td>
</tr>
<tr>
<td>Average duration of treatment (year)</td>
<td>4(1-6) ±2</td>
<td>3(1-5) ±2</td>
</tr>
</tbody>
</table>

**Table 2) Predictive value of gastrointestinal symptoms in RA and psoriasis**

<table>
<thead>
<tr>
<th>Variable</th>
<th>RA</th>
<th>Psoriasis</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of patients</td>
<td>85</td>
<td>50</td>
<td>Not sign.</td>
</tr>
<tr>
<td>Nausea and vomiting</td>
<td>20(23.5%)</td>
<td>10(20%)</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3) Predictive value of elevated liver enzymes in RA and psoriasis**

<table>
<thead>
<tr>
<th>Variable</th>
<th>RA</th>
<th>Psoriasis</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of patients</td>
<td>85</td>
<td>50</td>
<td>&lt;0.05 (Sign.)</td>
</tr>
<tr>
<td>Elevated liver enzymes</td>
<td>6(7%)</td>
<td>7(14%)</td>
<td></td>
</tr>
</tbody>
</table>
(Table 4) Predictive value of gender in RA and psoriasis

<table>
<thead>
<tr>
<th>Variable</th>
<th>RA</th>
<th>Psoriasis</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients with elevated liver enzymes</td>
<td>6</td>
<td>7</td>
<td>Not sign.</td>
</tr>
<tr>
<td>Female</td>
<td>4(66.6%)</td>
<td>3(42.8)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2(33.3)</td>
<td>4(57.1%)</td>
<td></td>
</tr>
</tbody>
</table>

(Table 5) Predictive value of age in RA and psoriasis

<table>
<thead>
<tr>
<th>AGE</th>
<th>Group</th>
<th>No. of patients with elevated liver enzymes</th>
<th>Average of age(year)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>R.A</td>
<td>6</td>
<td></td>
<td>46(24-60)±13</td>
<td>Not sign.</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>7</td>
<td></td>
<td>52(35-65)±12</td>
<td></td>
</tr>
</tbody>
</table>

(Table 6): Predictive value of BMI in RA and psoriasis

<table>
<thead>
<tr>
<th>BMI</th>
<th>Group</th>
<th>Number of patients with elevated liver enzymes</th>
<th>Average BMI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kg/m²</td>
<td>R.A</td>
<td>6</td>
<td>23(21-25)±2</td>
<td>&lt;0.05 (Sign.)</td>
</tr>
<tr>
<td></td>
<td>Psoriasis</td>
<td>7</td>
<td>28(21-38) ±3</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

The study showed that gastrointestinal problems like nausea, vomiting in RA group was 23.5% and in psoriasis group was 20% which was statistically not significant, in agree to study in 2006, where nausea and vomiting in psoriasis group (9.8) and in RA group was 13% which also statistically not significant. A significant rise in liver enzymes were seen in 6 patients (7%) with RA group and in 7 patients (14%) with psoriasis group, our study agrees with study in 2006 showed that significant rise in liver enzymes in psoriasis group (14.5%) and 7.5% in RA group. The study showed that the gender in RA group and psoriasis group not significantly correlated with hepatic toxicity in contrast to other study, one in 2006(20) other in 2004(34), both showed male in psoriasis group significantly more affected than male in RA group. The study showed no significant correlation of both the age, duration of MTX treatment with the level of liver enzymes abnormality in agree to a study in 2006(21) and other in 2004(22) The study showed that both BMI, cumulative dose, were significantly correlated with hepatic toxicity in psoriasis versus RA group in agree with study in 2004(23) The study showed a significant association between weekly dose of MTX with hepatic toxicity in psoriasis group versus RA group in contrast to study in 2004(22) The study showed a significant association between serum cholesterol level and hepatic toxicity in psoriasis group versus RA group in agree to study in 2006(24) but in contrast to previously reported studies(25--26) were total cholesterol level did not correlate with hepatic toxicity. This study showed no significant association between serum creatinine level and hepatic toxicity in psoriasis group versus RA group, a study in 2004(22) show that renal impairment, diabetes and obesity were significantly correlated with hepatic toxicity Current study show that liver enzymes (transaminases and ALP) both increased
in both groups (RA and psoriasis), although increase level of ALP more correlated with hepatotoxicity \textsuperscript{(27)}. The study recommended encouragement of life style modification for patients who are overweight or obese with psychological support especially for patients with psoriasis. Viral serology for HBS Ag and HCV as baseline because MTX therapy in patient with positive serology may enhance viral replication and result in fulminant hepatitis and Baseline lipid profile as hyperlipidaemia is an independent risk factor for MTX hepatotoxicity.

**Conclusion**

MTX related hepatic toxicity was more significant in patient with psoriasis than patient with RA. BMI, cumulative dose, weekly dose and serum cholesterol level are risk factor for hepatotoxicity in psoriasis group than in RA group.

**Acknowledgement**: The authors would like to thank the internal medicine department in Marjan Teaching Hospital in Babil/Iraq.

**Ethical Clearance**: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

**Conflict of Interest**: The authors declare that they have no conflict of interest.

**Funding**: Self-funding

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Scolicidal Activity of Zirconium Oxide (ZrO$_2$) nanoparticles Against Protoscolices of Hydatid Cysts

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Abstract

Hydatidosis is a sickness that affects human and farm animals. This disease is deemed as a public health problem in different regions of the world until nowadays. Surgical overlaps is the best way to treat the disease, while the risk of surgery lies in the possibility of cyst rupture and leakage of protoscolices and the recurrence of infection again, this prompted researchers to use scolicidal agents before surgery such as ethanol, plant extracts, to reduce parasite spread and recurrence of infection, recently researchers have been using nanoparticles as a scolicidal agent, like gold nanoparticles, silver nanoparticles, selenium nanoparticles, and others. This research aims to evaluate the fatal effect of zirconium oxide (ZrO$_2$) nanoparticles to protoscolices of hydatid cysts. The Protoscolices were collected from sheep livers infected with hydatid cyst disease. The protoscolices were treated with different concentrations (250, 500, 1000, 2000, and 4000 μg/ml) of ZrO2 NPs. The viability of protoscolices was determined by using an eosin staining method after 15, 30, and 60 min. The results showed that the concentrations of 1000, 2000, and 4000 µg/ml were significantly effective in the killing of protoscolices after 60 min., where the fatality rate of protoscolices was 49.6%, 52.7%, and 53.1% respectively when compared with the control group 38.5% (p<0.05).

Key words: ZrO$_2$, Zirconium oxide nanoparticles, protoscolices, Scolicidal, hydatidosis.

Introduction

Echinococcosis is a zoonosis, including two types of hosts, definitive (carnivores) and intermediate (wide range of mammalian species). The parasites of genus Echinococcus are small cestodes (1-6 mm) (1). The metacestodes of E. granulosus are cysts of different sizes filled with clear liquid and are called hydatids. The first discovery of hydatid disease dates back to the time of Hippocrates (2). Cystic echinococcosis (CE) is a growing health problem in various parts of the world, including the Middle East (3). The liver is the first organ in which the parasite settles and develops into the larval stage (hydatid cyst) (4). The germinal inner layer of hydatid cyst represents the origin of protoscolices, which are the infective form to the definitive host, and the source of secondary infection when naturally or experimentally released within mammalian tissues or peritoneal cavity (5, 6). Surgery and/or chemotherapy are a common treatment for CE, chemotherapeutic of this disease has been sophisticated in several animal model studies, and both albendazole and mebendazole are considered to have identical efficiency (7). Surgery is used in special cases depending on the characteristics of cysts, such as large cysts that contain multiple daughter cysts, single superficial cysts at risk of rupture, and cysts interlaced with biliary tract. PAIR (Percutaneous, Aspiration, Injection and Re-aspiration) is an alternative way to surgery, with minimum risks (8). Efforts were dedicated to finding out new protoscolicidal materials from plant sources, those efforts concentrated on some plant extracts that showed high effectiveness against CE (7). Due to the unavailability of effective treatment for CE, there is an urgent need to find this treatment, so that the nanotechnology-based materials may be useful in the cure of diseases (9). Nanotechnology is a technique that is concerned with the development and use of chemicals, devices, and systems of unfamiliar characteristics because of their small size (1-100 nm), in addition to their unique physicochemical properties which had great importance in many fields of research, including science and medicine (10).

ZrO$_2$ NPs have been studied extensively because of their unique mechanical, thermal, optical, and electrical properties (11). They have broad applications because
of remarkable biocompatibility, high strength, and low cost (12). ZrO2 NPs are also able to possess noticeable antimicrobial properties (13).

**Materials and Method**

1- **Collection of protoscolices.**

Samples of hydatid cysts were collected from livers of naturally infected sheep in a slaughterhouse in Baghdad/Iraq. The hydatid fluid was withdrawn from the cysts by using a 20 ml syringe and transferred into flasks. The fluid was left for enough time to enable all protoscolices to precipitate. The germinal layers of the cysts were also collected and placed in a petri dish and washed very well to assemble all protoscolices. The protoscolices were suspended with normal saline. The viability of protoscolices calculated according to the method of Smyth & Barrett (14).

2- **Preparation of nanoparticles suspension.**

Zirconium nanoparticles (29.8 nm) were purchased. Jeng & Swanson (15) method was adopted to prepare nanoparticles suspension with modulation, where 0.4g of nanoparticles were weighed and suspended in 100 ml of distilled water and sonicated for 20 minutes before use. Five concentrations of the ZrO2 NPs (250, 500, 1000, 2000, 4000) µg/ml were prepared according to the method of Napooni et al. (16) with some modifications.

3- **Solicidal activity of ZrO2 nanoparticles.**

The efficiency of five concentrations (250, 500, 1000, 2000, 4000) µg/ml of ZrO2 NPs were tested against protoscolices of hydatid cysts at different exposure times (15, 30, 60) min. 0.5 ml of protoscolices suspension (1000 ps) were mixed with 0.5 ml of each concentration of nanoparticles, then incubated at 37°C for 15, 30 and 60 min. Then the viability of Protoscolices calculated after exposure to each concentration/time.

4- **Statistical analysis**

The data were analyzed by using statistical software package IBM SPSS (version 25). The differences between experimental groups and control groups estimated by using ANOVA and T-tests at a significance level of 0.05.

**Results**

Fatality rate (%) of protoscolices after exposure to ZrO2 NPs at various concentrations following various exposure times as shown in Figure 1.

![Figure 1: Fatality rate (%) of protoscolices after exposure to ZrO2 NPs at various concentrations following various exposure times.](image-url)
* The difference between the experimental group and control group is statistical significance (p<0.05)

Discussion

The results of this study showed that the highest fatality rate of protoscolices was 49.6%, 52.7%, and 53.1% when treated with ZrO2 NPs suspension at concentrations 1000, 2000, 4000 µg/ml, respectively, after 60 min., and the difference between these groups and control group (38.5%) was statistically significant (p<0.05). Because of the importance of nanoparticles in medicine and science, the researchers studied its effects against some harmful organisms, especially bacteria and parasites, like the studies of Yalcinkaya et al. (17) and Kumaresan et al. (18), which proved the great effect of ZrO2 against E. coli, Bacillus subtilis, Salmonella typhi. Rahini et al. (19) studied the effect of green synthesis silver nanoparticles against protoscolices, and they found that the highest mortality rate was 90% (0.15 mg/ml, 120 min.). While the highest mortality rate was achieved when using the green synthesis gold nanoparticles (94%, 0.3 mg/ml, 120 min.) (20). Naponi et al. (16) have also used gold nanoparticles at the same concentration for the current study and after 5, 10, 20, 30, and 60 min., they proved that all concentrations had a potential effect against protoscolices, while the highest mortality rate reached 76% (4000 µg/ml/ 60 min.).

Conclusion

It was concluded from this study that the ZrO2 was effective in the killing of the protoscolices of hydatid cysts, and the highest mortality rate reached 53%. It can be used other concentrations to elevate the efficiency of ZrO2 or combine these nanoparticles with plant extracts or drugs. It is suggested to study the effect of ZrO2 against hydatid disease in vivo, to ensure its safety and possible use as a treatment against this disease in the future.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References


Molecular Study of Fimh Gene in Klebsiella Pneumoniae Isolated From Urinary Catheter Patients

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Abstract

This study aimed to isolate and diagnose K. pneumoniae from clinical specimens of urine from urinary catheterized patients and molecular Detection of FimH fimbrial adhesin in Klebsiella pneumonia in Najaf governorate from October 2018 to March 2019, which includes 40 clinical specimens (urine). The diagnosis of K. pneumoniae isolates was based on culture and biochemical characteristics as an initial diagnosis. The final diagnosis by the Vitek-2 compact system is automated besides the use of PCR technique to detect on fimH fimbrial adhesion gene.

The biochemical results showed that 40/40 isolates gave positive result of K. pneumoniae. These results were confirmed by Vitek showed that 40/40 were positive for K. pneumoniae isolated and PCR technique by using fimH gene where 14/40 were positive for K. pneumoniae isolated from urine urinary catheterized patients.

The study, which was conducted in the diagnosis of bacteria, concluded that the technique of compact Vitek-2 automated. The ability of bacteria to stick to the formation of biofilm was investigated by phenotypic method.

Keywords: K. pneumonia, fimH gene, urinary catheterized.

Introduction

Catheter-associated urinary tract infections (CAUTIs) are most frequent as a nosocomial infection with increased patient morbidity and health care costs. Klebsiella pneumoniae is a prominent opportunistic pathogen causing infection in 10% of the patients with urinary catheters. The catheter insertion provides site for bacteria attachment that is typical in Gram-negative enterobacteria (1, 2). Furthermore, about 30% of K. pneumoniae isolates are resistant to broad-spectrum antibiotics with many virulence factors that have been identified. Fimbrial adhesins play an important role in the bacteria pathogenicity that facilitate adherence to specific tissue surfaces. Type 1 fimbriae, especially FimH subunit, found in many members of Enterobacteriaceae and play an important role in UTI (3-5). Fimbriae are encoded by Fim gene cluster containing all fimbrial structure genes coding for repeating FimA subunits with an adhesin molecule (FimH) at the tip (6).

Material and Method

Patients and clinical specimens

A total of 40 urine samples were collected from urinary catheterized patients from different hospitals in Al-Najaf provenance from Oct. 2018 to March 2019.

Urine specimens were cultured on MacConkey and Blood agar figure (1), then inoculated at 37°C for 18-24 hours (7).
Identification of bacteria

It studied the colonial characteristics such as (shape, volume, color, borders and texture) and examined microscopically after staining with gram-stain (8) and biochemical test for diagnosis of *K. pneumoniae*. Finally, identification was performed with automated VITEK-2 compact system using G-ve ID cards.

Molecular study

Extraction of Genomic DNA

Genomic DNA was extracted by using boiling method DNA was extracted from colonies grown on agar plates by boiling method according to , taking colonies of bacteria grown on MacConkey agar plates were suspended in 300μl of Tris-EDTA Buffer buffer in Eppendorf tube, then vortex and boiling at 100°C for 15 min and immediately freeze at -20°C for 20 min to lyses the organisms and release the DNA, then centrifuged at 8000 xg for 5 min ,supematant transferred to new Eppendorf tube and stored at -20°C until used.

Polymerase Chain Reaction Protocol

The DNA extract of *K. pneumoniae*. Isolates were subjected to flageller gene genes listed in (Table 1) by using PCR. The protocol was used depending on Promega Biosystem manufacturer’s instruction. Single reaction (final reaction volume 20μl as in table (1). All PCR components were assembled in PCR tube and mixed by refrigerated microcentrifuge at 50 rcf for 10 second.
Table 1: Protocol of monplex PCR reaction mixture volumes

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Master mix</td>
<td>8µL</td>
</tr>
<tr>
<td>DNA template</td>
<td>5µL</td>
</tr>
<tr>
<td>Forward primers</td>
<td>1.5µL</td>
</tr>
<tr>
<td>Reverse primers</td>
<td>1.5µL</td>
</tr>
<tr>
<td>Deionized water (dd water)</td>
<td>4µL</td>
</tr>
<tr>
<td>Final volume</td>
<td>20 µL</td>
</tr>
</tbody>
</table>

**PCR Thermocycling Conditions**

The PCR tubes were placed on the PCR machine and the right PCR cycling program parameters conditions were installed as in table (2).

**Agarose Gel Electrophoresis:** According to (9).

Table 2: Amplification Conditions of genes were used by PCR reactions.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Initial denaturation</th>
<th>No. of cycles</th>
<th>Denaturation</th>
<th>Annealing</th>
<th>Extension</th>
<th>Final extension</th>
</tr>
</thead>
<tbody>
<tr>
<td>fimH</td>
<td>94°C for 4min.</td>
<td>35</td>
<td>94°C for 30sec.</td>
<td>52.9°C for 30sec.</td>
<td>72°C for 50sec.</td>
<td>72°C for 7min.</td>
</tr>
</tbody>
</table>

**Results and Discussion**

**Prevalence of *K. pneumoniae* specimens**

This study was conducted on 40 specimens from urine urinary catheterized patients during the period from October 2018 to March 2019, all these specimens 40 were inoculated on MacConkey and Blood agar medium as at 37°C for 18-24 hours (7).

**K. pneumoniae identification**

Morphologically characterization

The bacterial isolates obtained from clinical samples were identified initially according to cultural morphology, microscopic characteristics and biochemical tests. From those isolates, the cultural identification of *K. pneumoniae* was depended on the colonial morphology. Since the colonies of *K. pneumoniae* were grown on blood agar appears non-haemolytic smooth white colonies and red colour like shaped and smooth colonies when grown on the MacConkey agar, indicated that *K. pneumoniae* is able to ferment lactose sugar (Figure 4-1).

The results of biochemical tests that recorded in table (3) were considered as a complementary of the initial identification of *K. pneumoniae* isolates. The isolates confirm to general characteristics, isolates were negative for oxidase test. Urease production and Simmons citrate utilization and catalase test positive result. All the result (morphology and cultural) were identical with (10).

Table 3: The Biochemical features of *K. pneumoniae*

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidase test</td>
<td>-</td>
</tr>
<tr>
<td>Simmons Citrate</td>
<td>+</td>
</tr>
<tr>
<td>Urea hydrolysis</td>
<td>+</td>
</tr>
<tr>
<td>Catalase test</td>
<td>+</td>
</tr>
</tbody>
</table>

Upon detailed bacteriological investigation based on the morphological, cultural and biochemical tests were 40 isolates as tentatively identified as *K. pneumoniae*.

The final identification was performed with the automated VITEK-2 compact system using GN-ID cards.
which contained 47 biochemical tests and one negative control well (Appendix1). The results demonstrate that 40 isolates from urine urinary catheterized patients were confirmed as *K. pneumoniae* with ID message confidence level ranging between very good to excellent (Probability percentage 99).

**Virulence factors of *K. pneumoniae***

**Adherence Variation**

Biofilm forming ability is highly linked to bacteria swarming in, which is represented as an important virulent factor \((11, 12)\). All of the tested isolates showed \((2cm-8cm)\) swarming (increase in colony diameter) after 24 hrs of incubation. The rates of the migration were measured at 0, 6, 12, 18 and 24 hr. At 0 hr, *K. pneumoniae* isolates appeared with no migration. At 6 hr and 12 hr, *K. pneumoniae* isolates appeared with same rate of migration. After 18-24 hrs, most of the *K. pneumoniae* isolates covered the entire media surface. These results agree with the findings of \((12)\) who reported more than \(1.5Cm - 7.7Cm\) after 24 hrs of incubation.

**Molecular Study**

**Genomic DNA Extraction**

DNA Genomic was successfully extracted from *K. pneumoniae* isolates by using boiling method. The concentration and purity of extracted DNA were directly determined by spectrophotometry, extracted DNA purity ranged between \((1.8 - 2)\). Extracted DNA were confirmed and analyzed by gel electrophoresis.

**Molecular Detection of fimH gene of *K. pneumoniae***

The results showed that *fimH* gene was detected in 14 / 40 of *K. pneumoniae* isolates as in figure (2).

![Figure 2: PCR amplicon of *K. pneumoniae* fimH gene. Product size 688bp. Lane (L), DNA marker (100-bp ladder), Lanes (1 to 14) positive results.](image)

Fimbriae are assumed to play critical roles in attachment to epithelial cell surfaces. Binding to specific host receptors, fimbriae mediate the bacterial colonization, host cell signaling. Fimbrial adhesins determine the fate of the bacterial pathogen in the host as well as the progress of the corresponding disease process. Type-1 fimbriae also play an important role in deciding the virulence of the organism. Experiments conducted by Jaroni indicated that a mannose-resistant haemagglutinin was required for the attachment of Klebsiella to target cells \((13)\). The present result assumed important role of fimbriae 1 in attachment to epithelial cell surface (mediates the bacterial colonization) and deciding the virulence of the *K. pneumoniae*. The relationship between mannose – sensitive hemagglutinin (MSHA) or type 1 fimbriae and pathogenesis of bacteria was established from adherence of bacteria in mucous surfaces or epithelial cells of gastric tract \((14)\).

**Conclusions**

The use of Vitek-2 system, is necessary to confirm precise identification of this pathogen. Clinical isolates of *K. pneumoniae* possess number of virulence factor that associated with Urinary Catheter such as adhesion factors (biofilm). The *fimH* gene that encoded for adhesion factors (biofilm) was found almost in *K. pneumoniae* isolates.
Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-Funding

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Chemerin Level as a Marker in Preeclampsia and its Relation to the Disease Severity and Neonatal Outcome

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Abstract

Aim of study: To detect serum chemerin level in patient with preeclampsia and evaluate the association between maternal serum chemerin, disease severity and neonatal outcome.

Patients and Method: A case control study included 100 pregnant women with singleton pregnancy, gestational age of 20 weeks or more, normal fetal morphology, and absence of concomitant diseases, who were collected from inpatient during delivery was conducted in the Department of Obstetrics and Gynecology at Al-Yarmouk Teaching Hospital during the period from 1st of June 2018 till end of May 2019. They were divided into three groups (control, mild preeclampsia, and severe preeclampsia). Patients with history of chronic hypertension, diabetes mellitus, cardiovascular disease, neurological disorder, renal impairment, or premature rapture of membrane were excluded from this study. blood sample was taken from all patients and sent for human chemerin assay. After delivery, birthweight of baby, APGAR scores at one and five mints, neonatal intensive care unit and adult intensive care unit admission, and hospitalization time were also noted.

Results: There were no statistically significant differences between the study groups in age, BMI level, and parity. Chemerin level was significantly elevated in patients with severe preeclampsia (435.06 ng/ml) and mild preeclampsia (227.49 ng/ml) than that in non-preeclamptic patients (202.6 ng/ml). It was negatively correlated with each of gestational age, birth weight, Apgar score at one and five minutes. While it was positively correlated with admission’s duration. Serum chemerin > 228.5 ng/ml is predictive for diagnosis of preeclampsia and level > 380.9 ng/ml is indicator for severe preeclampsia.

Conclusion: Chemerin may play a role in the pathogenesis of preeclampsia as maternal serum chemerin level was significantly higher in patients with preeclampsia

Keywords: Preeclampsia, chemerin, APGAR score, birthweight, Iraq

Introduction

Preeclampsia (PE) is a syndrome that chiefly includes the new onset of hypertension and either proteinuria or signs of other end-organ dysfunction (e.g. hepatic abnormality, pulmonary edema, thrombocytopenia) (1). PE affect between 3% and 5% of all pregnancies and account for more than 60,000 maternal and 500,000 fetal deaths per year worldwide (2). It is one of the most important causes of maternal, perinatal, and fetal morbidity and mortality in the world (3). Deficient spiral artery remodeling, placental ischemia, release of mediators into the maternal circulation, systemic endothelial dysfunction, inflammation, and consequent increased vascular constriction are biological mechanisms that contribute to preeclampsia (4, 5). Additionally, abnormal placentation, imbalance of angiogenesis regulators, and maternal immune
maladaptation are other possible factors associated with preeclampsia (6). Moreover, the future risk of vascular and metabolic disease is significantly increased after a preeclamptic pregnancy (7). However, the pathogenesis of this life-threatening condition remains unclear (8). The placenta is thought to be a major source of endogenous nitric oxide (NO) during pregnancy. Endothelial NO, which is synthesized by endothelial NO synthase (eNOS), is an important regulator of blood flow and vasomotor tone via its inhibition of smooth muscle contraction. Thus, it is hypothesized that NO–eNOS system abnormalities are associated with the onset of preeclampsia (9, 10). Chemerin, named also as tazarotene-induced gene protein 2 or retinoic acid receptor responder protein 2, is a novel adipocytokine that is mainly expressed in adipocytes, liver, placenta, and ovaries (11). Evidence has been presented that chemerin is linked to facets of the metabolic syndrome in vitro and in vivo (12) hence this adipokine is correlated with insulin resistance and body fat accumulation (13). Hypertension, coronary disease (14), diabetes mellitus (15), atherosclerosis (16), obesity and metabolic syndrome (17) are also associated with chemerin. Although its specific biological functions are controversial, chemerin may play a role in the pathogenesis in preeclampsia. The aim of this study is to determine whether serum chemerin concentrations are elevated in preeclamptic women and whether serum chemerin levels differ according to severity of preeclampsia and to evaluate the association between maternal serum chemerin and neonatal outcome.

Patients and Method

Study design, setting: This is a case control study that was conducted in the Department of Obstetrics and Gynecology at Al-Yarmouk Teaching Hospital during the period from 1st of June 2018 till end of May 2019.

Study Population and Sample Size: The study included 100 pregnant women with singleton pregnancy, gestational age of 20 weeks or more, normal fetal morphology, and absence of concomitant diseases, who were collected from inpatient during delivery in labor room. They were divided into three groups:

- Severe Group: Included 33 pregnant women who had diagnosed with severe PE.
- Mild Group: Included 33 pregnant women who had diagnosed with mild PE.
- Control Group: Included 34 pregnant women with uncomplicated pregnancy who were selected after matching for age and gestational age of another one in the other two groups after proof that she was normotensive by history, examination and investigation.

Mild PE is diagnosed when hypertension with two readings (separated by 4-6 hrs. apart) of systolic blood pressure ≥140 mmHg and / or diastolic pressure ≥90 mmHg. Another characteristic feature of mild PE is the development of proteinuria ≥300 mg / 24 hrs. (18). Severe PE can be identified by presence of sustained elevation in blood pressure, systolic blood pressure ≥160 mmHg or diastolic blood pressure ≥110 mmHg, proteinuria of >2 gm in a 24-hrs urine specimen, serum creatinine of >1.2 mg/dl, platelets of <100,000/dl, increased lactate dehydrogenase, elevated serum transaminase levels, persistent headache, and oliguria (urinary output<400 ml/24 hours). In addition, any patient with cerebral or visual impairment, persistent epigastric pain, pulmonary edema or cyanosis, impaired liver function, or thrombocytopenia (platelet count less than 100,000/ml) was diagnosed with severe PE (3). Pregnant women with history of chronic hypertension, diabetes, cardiovascular disease, neurological disorders, renal impairment, or with premature rapture of membrane were excluded from this study.

Data collection: All patient told about the nature of the study and verbal consent was taken from them. Information about maternal age, gestational age, parity, gravidity, mode of delivery, previous history of preeclampsia, family history of any previous medical history. Then both group undergo to general examination, vital signs (systolic and diastolic blood pressure), abdominal and obstetric examination, laboratory investigation and sonographic examination. Then, from all study patients, we took 10 ml blood sample which was divided into two equal parts, the first one was sent to our hospital laboratories for CBC, blood group and Rh, blood sugar, b. urea, s. creatinine, SGOT, SGPT, coagulation profile and platelet count. In the other part, serum was separated by centrifugation at 2,500 rpm for 10 min and frozen at -70 °C. The serum chemerin levels were measured by enzyme linked immunosorbent assay according to the manufacturer’s instructions. The lowest level of human chemerin that can be detected by this assay is 31.2 ng/ml.

After delivery, birthweight of baby, APGAR scores at one and five mints, neonatal intensive care unit
(NICU) and adult intensive care unit (AICU) admission, and hospitalization time were also noted.

**Statistical analysis**

The data analyzed using Statistical Package for Social Sciences (SPSS) version 25. They presented as mean, standard deviation and ranges. Categorical data presented by frequencies and percentages. Analysis of Variance (ANOVA) (two tailed) was used to compare the continuous variables accordingly. Pearson’s correlation test (r) was used to assess correlation between continuous variables accordingly. Receiver operating characteristic (ROC) curve analysis was used for prediction of chemerin level as diagnostic of preeclampsia. A level of P – value less than 0.05 was considered significant.

**Results**

In this study, 100 pregnant women were enrolled. The age was ranging from 17 to 40 years with a mean of 25.9 ± 6.13 years. Regarding general characteristics, there were no statistical significant differences (P ≥ 0.05) between the study groups in age, BMI, and parity. Concerning blood pressure, SBP and DBP were significantly higher in severe group than that in mild and control groups (169.34 versus 148.93 and 123.81 mmHg, P= 0.001; and 107.1 versus 95.9 and 75.53 mmHg, P= 0.001 respectively).

About investigation, AST, ALT, s. urea and chemerin level were significantly higher in severe group than that in mild and control groups (155.06 versus 45.48 and 30.37 U/l, P= 0.001; 126.0 versus 35.36 and 25.71 U/l, P= 0.001; 31.65 versus 25.0 and 25.78 mg/dl, P= 0.001; and 435.06 versus 227.49 and 202.6 ng/ml, P= 0.001 respectively). Mean of platelet count was significantly lower in severe group than that in mild and control groups (101.65 versus 149.21 and 198.09, P= 0.001)

No statistical significant differences (P ≥ 0.05) between the study groups in WBC and s. creatinine as shown in table (1).

**Table 1: Comparison between study groups by general characteristics, blood pressure, and investigation**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Severe Group Mean ± SD</th>
<th>Mild Group Mean ± SD</th>
<th>Control Group Mean ± SD</th>
<th>P - Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>General characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal age (Year)</td>
<td>24.45 ± 6.3</td>
<td>25.0 ± 5.0</td>
<td>26.23 ± 6.5</td>
<td>0.564</td>
</tr>
<tr>
<td>BMI (Kg/m2)</td>
<td>28.43 ± 3.8</td>
<td>26.61 ± 3.8</td>
<td>29.35 ± 4.1</td>
<td>0.093</td>
</tr>
<tr>
<td>Parity</td>
<td>1.36 ± 1.7</td>
<td>1.37 ± 1.7</td>
<td>1.35 ± 1.7</td>
<td>0.279</td>
</tr>
<tr>
<td>Blood pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>169.34 ± 17.3</td>
<td>148.93 ± 6.2</td>
<td>123.81 ± 10.1</td>
<td>0.001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>107.1 ± 20.1</td>
<td>95.9 ± 5.3</td>
<td>75.53 ± 7.8</td>
<td>0.001</td>
</tr>
<tr>
<td>Investigation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC (10 9/l)</td>
<td>12.32 ± 2.8</td>
<td>11.17 ± 3.9</td>
<td>10.62 ± 3.4</td>
<td>0.127</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>155.06 ± 210.9</td>
<td>45.48 ± 7.4</td>
<td>30.37 ± 6.6</td>
<td>0.001</td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>126.0 ± 148.6</td>
<td>35.36 ± 7.5</td>
<td>25.71 ± 6.2</td>
<td>0.001</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>31.65 ± 10.0</td>
<td>25.0 ± 4.2</td>
<td>25.78 ± 5.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.71 ± 0.23</td>
<td>0.77 ± 0.16</td>
<td>0.81 ± 0.16</td>
<td>0.126</td>
</tr>
<tr>
<td>PLT Count (10 9/l)</td>
<td>101.65 ± 19.5</td>
<td>149.21 ± 26.6</td>
<td>198.09 ± 37.2</td>
<td>0.001</td>
</tr>
<tr>
<td>Chemerin level (ng/ml)</td>
<td>435.06 ± 55.4</td>
<td>227.49 ± 57.4</td>
<td>202.6 ± 21.1</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Receiver operating characteristic (ROC) curve analysis was constructed for chemerin level as indicator of preeclampsia. As shown in figure (1) and table (2), the cut point of chemerin level was 228.5 ng/ml, so s. chemerin > 228.5 ng/ml is predictive for diagnosis of preeclampsia indicating significant association between higher level of chemerin and diagnosis of preeclampsia.

![ROC Curve / Chemerin / AUC=0.9523](image1)

**Figure 1: ROC curve for chemerin as a marker of preeclampsia**

**Table 2: Diagnostic accuracy for test of preeclampsia**

<table>
<thead>
<tr>
<th>Chemerin level (ng/ml)</th>
<th>Cut-off value</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>228.5</td>
<td>89.3%</td>
<td>94.1%</td>
<td>96.7%</td>
<td>82%</td>
<td>91%</td>
</tr>
</tbody>
</table>

ROC curve analysis was constructed again for chemerin level as diagnostic for severity of preeclampsia. As shown in figure (2) and table (3), the cut point of chemerin level was 380.9 ng/ml, so s. chemerin > 380.9 ng/ml is indicator for severe preeclampsia indicating significant association between higher level of chemerin and diagnosis of severe preeclampsia.

![ROC Curve / Chemerin / AUC=0.9900](image2)

**Figure 2: ROC curve for chemerin level as a marker of severe preeclampsia**

**Table 3: Diagnostic accuracy for test of severe preeclampsia**

<table>
<thead>
<tr>
<th>Chemerin Level (ng/ml)</th>
<th>Cut-off value</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>380.9</td>
<td>93.9%</td>
<td>100%</td>
<td>100%</td>
<td>97.1%</td>
<td>98%</td>
</tr>
</tbody>
</table>
Chemerin level was negatively correlated with each of gestational age, birth weight, Apgar score at one and five minutes. While it was positively correlated with admission’s duration, and these correlations were demonstrated in table (4).

**Table 4: Correlation between level of chemerin and certain obstetric and neonatal outcomes of the study groups**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Chemerin Level (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
</tr>
<tr>
<td>Gestational age (Weeks)</td>
<td>-0.711</td>
</tr>
<tr>
<td>Birth Weight</td>
<td>-0.714</td>
</tr>
<tr>
<td>Apgar Score at 1 Mint</td>
<td>-0.615</td>
</tr>
<tr>
<td>Apgar Score at 5 Mint</td>
<td>-0.709</td>
</tr>
<tr>
<td>Duration of Admission</td>
<td>0.547</td>
</tr>
</tbody>
</table>

**Discussion**

Recently, it has been reported that circulating chemerin concentrations were strongly correlated with the key markers of the metabolic syndrome, including insulin resistance, hyperlipidemia, and high blood pressure. In the current study, we found that maternal serum chemerin level was significantly higher in severe and mild preeclamptic patients compared to healthy pregnant women, and elevated serum chemerin level (>228.5 ng/ml) indicated preeclampsia with 89.3% sensitivity and 94.1% specificity and > 380.9 ng/ml indicated severe preeclampsia with 93.9% sensitivity and 100% specificity. These result was agreed with results conducted by Cetin et al study 2017 (3), Xu QL et al study 2014 (19), Wang L et al study 2015 (8), Duan DM et al study 2012 (20), and Stepan H et al study 2011 (21) when they all showed that serum chemerin was significantly higher in patients with preeclampsia than that of healthy pregnant women. These findings indicate that differential expressions of chemerin may be responsible for pathological changes in patients with preeclampsia. Chemerin was identified in maternal circulation during pregnancy. Placenta releases the major part of chemerin during the gestational period and also it plays a critical role in controlling/contributing to metabolic processes (22, 23). The signaling pathway between high expression of chemerin and its receptor CMKLR1 is the common pathogenic factor of obesity, diabetes, hypertension and metabolic syndrome (24).

In this study, chemerin level was negatively correlated with each of gestational age, birth weight, Apgar score at one and five minutes, while it was positively correlated with admission’s duration. This is similar to studies conducted by Cetin et al 2017 (3), and by Duan DM et al 2012 (20). Additionally, preeclampsia can lead to higher frequency of induced labor, neonatal respiratory difficulties, and increased frequency admission to neonatal intensive care unit (25). In conclusion, maternal serum chemerin level is significantly increased in preeclampsia, especially in severe preeclampsia. Larger studies and work are needed to better determine the mechanisms by which serum chemerin is increased in preeclampsia.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** Self-funding

**References**


Production, Analysis and Optimization of Inulin Produced from *Pseudomonas fluorescens*

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**Abstract**

Inulin is a natural polysaccharide produced from organisms and microorganism. Inulin is a type of fructan that α-amylase cannot digest it and cannot hydrolysis by hydrolytic enzymes. It was used to get non-fat fermented milk and it’s a type of prebiotics that induce growth and activity of probiotics bacteria, thus improving the health. *Pseudomonas fluorescens* have the capacity to produce inulin when grown in media supplied with sucrose. Twenty-two of bacterial isolates were belong to *Pseudomonas fluorescens* depending on structural features, microscopic checking, biochemical analysis and flourescent pigments that produced on King B medium. Ten isolates of *P. fluorescens* had strongly degree of mucous growth on solid production medium, the gummy and mucous manifestation on agar medium containing sucrose were give rise to inulin production. Inulin produced from *P. fluorescens* was analyzed by Fourier Transform Infrared Spectroscopy to detect functional groups which it was (C-O, CH, OH and C=O) and by Thin Layer Chromatography to determine its components of monosaccharide. Inulin distinguished as dark spot on white background and the Rf of it was (0.58). The best product of inulin were in production salt agar medium containing 20% sucrose, pH =7, Temperature =37°C without nitrogen sources and the inoculum size 1%, it was (3.2 gm ∕100 ml). The effects of bacterial inulin on the growth of *Saccharomyces cerevisiae* was studied by culturing it in medium supplemented with 3% of bacterial inulin at 30°C for 48 hrs. The results showed there was no remarkable effect of inulin on *Saccharomyces cerevisiae* growth in comparison to control.

**Keywords:** Inulin, Fructan, Levan, *Pseudomonas fluorescens*, *Saccharomyces cerevisiae*, Probiotic, Prebiotics, Biochemical test.

**Introduction**

Inulin is a natural polysaccharide produced from organisms and microorganism (¹, ²). It is a type of fructan that α-amylase cannot digest it and cannot hydrolysis by hydrolytic enzymes (³, ⁴). Inulin have protective effect on the survival and activity of lactic acid bacteria when storage and use it as final product (⁵, ⁶), and it was stimulated the growth of probiotic bacteria thus improving the health (⁷). In food technology inulin use to improve body mouthful, as stabilizers, fat replacers, and flavour enhacers (⁸, ⁹), *Pseudomonas fluorescens* capable for adaptation at different environments by extracellular substances (¹⁰). Living micro-organisms and its products are widely used for therapeutic purposes, *Saccharomyces cerevisiae* also possess some medicinal efficiency, and the beneficial properties of it are well documented (¹¹, ¹²).

At present day many pharmaceutical preparations with microorganisms products are commercially available (¹³, ¹⁴).

The aim of this study was to seek the capacity of local isolate of *Pseudomonas fluorescens* to produce inulin in different conditions and the effect of inulin on *Saccharomyces cerevisiae* growth.

**Materials and Method**

**Samples collection**

Thirty-five of different food samples were collected from different local markets in Baghdad governorate in sterilized utensil and imparted to the laboratory until using.

**Sorting and Identity of bacteria**

Half-gram were taken from specimen and 4.5 ml of sterilized peptone water were added, next dilutions were done, MacConkey agar was prepared and inoculated with 100 μl from the adequate dilution (1×10⁷), incubated at 37°C for 24 hrs. Fluorescing colonies were taken
and streaked again on the same agar medium several times till a pure culture was obtained. Bacterial isolates were identified by using selective medium (King B medium), structural features, microscopic checking and biochemical analysis (15).

**Checking of Inulin generating isolates**

Purified bacterial isolates were activated in Brain Heart Infusion broth (BHI), after incubation periods (0.1 ml) of culture suspension was streaking on production medium (3gm KH2PO4, 3gm K2HPO4, 0.5 gm MgSO4.7H2O, 20% w/v of sucrose and 2% agar-agar), incubated at 37°C for 24hrs. Mucoid consistence of bacterial colonies gave marked of inulin production.

**Quantitative checking in liquid medium**

The highly mucous isolates were selected, 10 ml of (BHI) broth were prepared and inoculated with bacterial isolates then incubated for 18hrs at 37°C, after incubation periods 100 ml of mineral broth (supplied with 20% of sucrose) was cultivated with 1ml of isolates, incubated for 24hrs, 37°C. Centrifuge were using (6000rpm, 30 minutes) for extracting inulin by mixing the cell free supernatant with ethanol at rate (1:4) and allowed to stand overnight, the aqueous layer was removed and the layer of inulin was collected in sterilized petri-dish and dried at 60°C.(16).

**Diagnosis of Inulin:**

a) **Fourier Transform Infrared Spectroscopy (FTIR):** Inulin dried weight was analyzed by using the crystal of potassium bromide (KBr) at rate 1:10 (w/w) (17)

b) **Thin Layer Chromatography (TLC):** This method was done according to (Shida et al. (2002)) (18) as following:

1. Inulin was dissolved (0.01gm) in 1N HCL and incubated at 70°C for 3 hrs.

2. About 10 µl of this suspension was taken and spotted plentiful of time away from the below end of TLC plate.

3. Sucrose, Fructose and Glucose solutions were destined and spotted in the same manner and they used as marker.

4. The plate of TLC placed in a closed jar containing separation system (butanol: propanol: D.W.: acetic acid at proportion of (7:5:4:2,v:v:v:v), until spread through the plate at 15 cm.

5. TLC taken and drying up.


7. Inulin demonstration as dark spot and the space of it were determination.

8. Relative flow (Rf) estimation as the following:

Distance of the sample mobilized across the plate / Distance of the solvent (20).

**Influence of some factors on inulin product**

1. **Carbon**

   A. Production broth (supplied with 20% of glucose and lactose) were prepared, inoculated with bacterial growth culture, incubated for 24hrs, 37°C.

   B. Extraction of inulin and determination of dry weight.

   C. Comparison with inulin dry weight which extracted from mineral broth containing 20% sucrose.

2. **Nitrogen**

   A. Production broth with best carbon source were prepared with addition of 1% (yeast extract and peptone), inoculated with bacterial growth culture, incubated for 24hrs, 37°C.

   B. Extraction the product and determination the weight.

3. **pHs**

   A. Production broth with best carbon source prepared at pHs (5, 6, 7, 8, 9 and 10), inoculated with bacterial growth culture, incubated for 24hrs, 37°C.

   B. Extraction the product and determination the weight.

4. **Temperature**

   A. Production broth with best (carbon source and pH) prepared, inoculated with bacterial growth culture, incubated for 24hrs, at (37, 45 and 50°C).

   B. Extraction the product and determination the
weight.

The effect of inulin on *Saccharomyces cerevisiae* growth

In order to investigate *Saccharomyces cerevisiae* growth in existing inulin, *S. cerevisiae* was cultivated in potato dextrose broth, incubated at 30°C for 48hrs then centrifugation for 15 min with 2500×g at 4°C. The precipitate was taken and washed by (PBS) (0.1 M phosphate buffer pH 7.4, 0.9% saline) and re suspended in PBS.

The suspension was cultured in medium supplied with 3% inulin, incubated at 30°C for 48hrs. The turbidity of cultured medium was measured at 600 nm for up to 48 hrs and compared with control cultured medium free from inulin (21).

Results and Discussion

Sorting and Identity of *Pseudomonas*:

Thirty-five of different food samples were collected from different local markets in Baghdad governorate. Twenty-tow of this isolates were diagnosed as *Pseudomonas* according to structural features and microscopic checking (17). Fluorescent producing on King B medium and Microscopic examination showed Gram-negative bacilli, non spore former bacteria and biochemical analysis showed (urease+, oxidase +, Gelatine hydrolysis +, Catalase +, Starch hydrolysis -) these results showed that they were identified as strains of *Pseudomonas fluorescens* (22).

Checking of Inulin generating isolates

*Pseudomonas fluorescens* streaking on production medium; for checking their capability to generate mucus manifestation as marking for Inulin production.

Inulin production was varied from species to species. The gummy and mucous manifestation is coming from manufacturing of polysaccharide (23).

Quantitative checking in liquid medium

Ten isolates of *P. fluorescens* with strongly degree of mucous growth had selected for checking their capability to generate inulin in broth medium that supplied with 20% of sucrose.

The highly mucous appearance of these isolates were taken for creation of inulin in mineral salt broth and the highly product was 3.2 gm/100 ml.

Diagnosis of Inulin:

a) Fourier Transform Infrared Spectroscopy (FTIR): Inulin generated by *P. fluorescens* was analyzed by FTIR spectroscopy to detect the effective structure of inulin.

The results showed the presence of C-O stretching group in 1122.49 cm⁻¹, bending group CH,OH in 1336.58 cm⁻¹ and 1434.23 cm⁻¹, stretching C=O in1649.02 cm⁻¹, stretching CH in 2891.10 cm⁻¹ and 2931.60 cm⁻¹, stretching OH in 3367.48 cm⁻¹ and 3431.13 cm⁻¹ as showed in figure (1). All these groups (C-O, CH, OH and C=O) are functional groups found in carbohydrates (17),

Figure 1: FT-IR for inulin production from *P. fluorescens*
Kazim AR. (2015) \(^{17}\) reported that polysaccharide extracted from \textit{Pseudomonas} does not contain lipids or nucleic acid in structures.

b) Thin Layer Chromatography (TLC): Inulin production from \textit{P. fluorescens} was analyzed by TLC chromatography to determine its components of monosaccharide.

Inulin extracted from \textit{P. fluorescens} was hydrolyzed with HCL before application on TLC and standard sugars (glucose, fructose and sucrose) were prepared and used as marker.

These sugars distinguished as dark spot on TLC plate when using TLC diagnosis solution (ethanol:H\textsubscript{2}SO\textsubscript{4} at proportion of 9:1, v:v.) as showed in figure (2).

Rf of these sugars (inulin, fructose, sucrose and glucose) were estimated according to Ghosh, S. and Chandra, A. (1980) \(^{20}\) and the results were (0.58, 0.59, 0.57 and 0.55) respectively.

Rf for the same compound differed according to (separation system, diagnosis solution and type of solvent) \(^{24}\).

![Figure 2: TLC for Inulin production from \textit{P. fluorescens}: Note: (1) inulin, (2)fructose, (3)sucrose and (4)glucose.](image_url)

**Influence of some factors on inulin product**

1. **Influence of carbon**

   Inulin product may be Influence by the (type, kind and concentration) of carbon substance.

   For investigation the influence of carbon, production medium supplied with 20\% of glucose, lactose and sucrose inoculated with \textit{Pseudomonas flourescens} and incubated for 24hrs, 37\(^{0}\)C.

   From study of the results, the best product of inulin from \textit{Pseudomonas flourescens} was obtained from production medium supplied with sucrose (3.2 gm/100 ml) and the minimum productivity was in existence of glucose (0.2gm/100 ml) as shown in figure(3).

   The amount of inulin was varied and the highest inulin production was produced when \textit{Pseudomonas flourescens} was cultivated in medium containing 20\% of sucrose.

   Microorganisms catabolized polysaccharide such as sucrose by levansucrase enzyme which degradation of sucrose \(^{15}\).

![Figure 3: Influence of carbon sources on the productivity of inulin from \textit{Pseudomonas flourescens}](image_url)

2. **Influence of nitrogen**

   By studying the results showed that there was reducing in productivity of inulin when supplying production medium with 1\% of yeast extract and peptone.

   The productivity decrease from (3.2 gm/100ml) to (2.1 gm/100) when supplied production medium with yeast extract and to (1.8 gm/100ml) in the present of peptone in medium as shown in figure (5).

   Microorganisms required nitrogen to complete the metabolic pathway, also nitrogen enhances and increase the microorganism growth; these increasing in growth may be decreased the production of inulin.

   The effect of yeast extract in production medium was studied previously, rising concentration of yeast extract responsible for an increase in the \textit{Zymomonas mobilis} and reduction the productivity of inulin, yeast extract also improves and enhancement of substrate consumption, increasing in consumption of substrate verified only a small part of consumed sugar and converted it to Inulin \(^{25}\).
3. **Influence of pHs**

From the results revealed; inulin productivity was varied with pH of production medium, the productivity decrease in pHs less than 7 and more than 7 as showed in figure (5).

Inulin is chemically stable in a natural and alkaline environment and its stability decreases in an acidic environment (25).

4. **Influence of temperature**

It was revealed that the best productivity of inulin at 37°C (3.2 gm/100ml) and the productivity was decrease at temperature above than 37°C, it was 2.1gm/100ml at 45°C and 0.1gm/100ml, figure (6).

From the result, the best temperature for the productivity was 37°C and at this temperature enzyme responsible for inulin may be synthesis.

Fructosyltransferase enzymes (FTFs) is bacterial enzymes responsible for synthesis of inulin and frctun from sucrose in three reactions (25).
**Fungal Growth study**

In order to investigate *Saccharomyces cerevisiae* growth in existing inulin, *S.cerevisiae* suspension was cultured in medium supplied with 3% inulin, incubated at 30°C for 48hrs. The turbidity of cultured medium was measured at 600 nm for up to 48 hrs and compared with control cultured medium free from inulin.

From the results of OD, (OD of control (2.8278), OD of test medium supplied with 3% inulin was 3.0158) there was no remarkable effect of inulin on *S.cerevisiae* growth when compared with the growth of control. *S.cerevisiae* had not enzymes responsible for degradation of inulin and used it as carbon sources.

Inulinases hydrolysis of inulin to glucose and fructose; already these molecules can be used as carbon sources to produce many beneficial products for microorganisms.

**Conclusion**

These studied seeking for the capacity of inulin production from local isolate of *Pseudomonas fluorescens*. The results showed no remarkable effectiveness of inulin on the proliferation of *Saccharomyces cerevisiae* compared with control.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.


Epidemiology of Hepatitis B and C in Al-Muthanna Province

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Abstract

This study systematically reviewed and synthesized available records of hepatitis B and C prevalence in Al-Muthanna province through the last five years from early 2014 till the end of 2018 in Women and Children Teaching Hospital at Al-Muthanna province. The study recorded high prevalence of hepatitis virus at this region, most of the patient were females and HCV was the most prevalent between them, the year of 2016 recorded the highest infection rate. Conclusion: High rate of Hepatitis C virus infection among of thalassemia patients

Keywords: hepatitis, blood donor, thalassemia

Introduction

Hepatitis is an inflammation of the liver, most commonly caused by a viral infection. There are 5 main hepatitis viruses, referred to as types A, B, C, D and E. These five types are of greatest concern because of the burden of illness and death they cause and the potential for outbreaks and epidemic spread. In particular, types B and C lead to chronic disease in hundreds of millions of people and, together, are the most common cause of liver cirrhosis and cancer. Hepatitis A and E are typically caused by ingestion of contaminated food or water. Hepatitis B, C and D usually occur as a result of parental contact with infected body fluids. Common modes of transmission for these viruses include receipt of contaminated blood or blood products, invasive medical procedures using contaminated equipment and for hepatitis B transmission from mother to baby at birth, from family member to child, and also by sexual contact.

The most common diseases that are transmitted through blood are hepatitis B and hepatitis C viruses. Infections with hepatitis B virus (HBV) and hepatitis C virus (HCV) are a worldwide public health problem. In Iraq, viral hepatitis prevention and control program was started during early seventies.

The transmission of HCV is primarily through exposure to infected blood. Risks for transmission include blood transfusion before 1992, intravenous drug use, high risk sexual activity, solid organ transplantation from an infected donor, occupational exposure, hemodialysis, household exposure, birth to an infected mother, and intranasal cocaine use.

Review

Hepatitis B and C

Hepatitis B virus is a member of the Hepadnavirus family. The virus particle, called Dane particle (virion), consists of an outer lipid envelope and an icosahedral nucleocapsid core composed of protein. The nucleocapsid encloses the viral DNA and a DNA polymerase that has reverse transcriptase activity similar to retroviruses.

Viral infection by hepatitis B virus (HBV) causes many hepatocyte changes due to the direct action of a protein encoded by the virus, HBx, and to indirect changes due to a large increase in intracellular reactive oxygen species (ROS) after infection. HBx appears to dysregulate a number of cellular pathways. HBx causes dysregulation in part by binding to genomic DNA, changing expression patterns of miRNAs, affecting histone methyl transferases, binding to SIRT1 protein to activate transcription, and cooperating with histone methylases and demethylases to change cell expression patterns.

HBx is partly responsible for the approximate 10,000-fold increase in intracellular ROS upon chronic HBV infection. Increased ROS can be caused, in part, by localization of HBx to the mitochondria where HBx decreases the mitochondrial membrane potential, in addition, another HBV protein, HBsAg, also increases
ROS through interactions with the endoplasmic reticulum\(^{(10)}\).

Hepatitis C (originally “non-A non-B hepatitis”) is caused by hepatitis C virus (HCV), an RNA virus of the family Flaviviridae. HCV can be transmitted through contact with blood (including through sexual contact if the two parties’ blood is mixed) and can also cross the placenta. Hepatitis C usually leads to chronic hepatitis, culminating in cirrhosis in some people. It usually remains asymptomatic for decades. Patients with hepatitis C are susceptible to severe hepatitis if they contract either hepatitis A or B, so all persons with hepatitis C should be immunized against hepatitis A and hepatitis B if they are not already immune, and avoid alcohol. HCV viral levels can be reduced to undetectable levels by a combination of interferon and the antiviral drug ribavirin. The genotype of the virus is the primary determinant of the rate of response to this treatment regimen, with genotype 1 being the most resistant. Hepatitis C is the most common chronic blood-borne infection in the United States\(^{(11)}\).

**Material and Method**

The results of the last five years were collected from (Al-Muthanna Women and Children Teaching Hospital) and organized in tables and charts based on gender, age, year of injury, accompanying diseases and residential areas.

**Results and Discussion**

Thirty three cases were identified through the last five years 13 (39%) of them were infected with HBV and 20 (61%) were infected with HCV, this indicates that the type C was more prevalent during these years. 85% of infection were females as the results were collected from delivery hospital.

<table>
<thead>
<tr>
<th>Table (1): Distribution of HBV and HCV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Item</strong></td>
</tr>
<tr>
<td>IgG ELISA</td>
</tr>
<tr>
<td>HBV</td>
</tr>
<tr>
<td>HCV</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Genus</strong></td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td><strong>Year of infection</strong></td>
</tr>
<tr>
<td>2014</td>
</tr>
<tr>
<td>2015</td>
</tr>
<tr>
<td>2016</td>
</tr>
<tr>
<td>2017</td>
</tr>
<tr>
<td>2018</td>
</tr>
<tr>
<td><strong>Region</strong></td>
</tr>
<tr>
<td>Rural</td>
</tr>
<tr>
<td>Urban</td>
</tr>
<tr>
<td><strong>Accompanying disease</strong></td>
</tr>
<tr>
<td>Thalasemia</td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
</tbody>
</table>

The age group (21-30) years was the most infected\((39.3\%)\) because it is the appropriate age for maturation and pregnancy and women are eligible for delivery in this age group, also the hospital was especially for women and children, while the age group \((≥10)\) years recorded the lowest rate, the CDC also notes that infections are rising among women of childbearing age, while the virus is not always transmitted from a pregnant woman to her baby, it is possible: About 6 infants in 100 born to mothers with the virus are infected\(^{(12)}\). Most cases were rural areas \((52\%)\) due to the use of drug injection is common. The year of 2016 recorded the
highest infection rate (36.3%) comparing with the year of 2015 which recorded no infections.

Fig (1): Distribution of HBV and HCV according to years of diagnosis

Three of the HCV cases had thalassemia as accompanying disease as hepatitis C virus (HCV) is the major cause of post-transfusion hepatitis infection (PTH). Patients with thalassemia major are at high risk of HCV(13).

Fig (2): Relation of thalassemia with type of hepatitis

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**Conflict of Interest:** The authors declare that they have no conflict of interest.

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**References**

11. CDC. Hepatitis C Information For the Health Professional”. 2010.
The Impact of Congenital Heart Diseases on Growth Parameters in Children and Their Correlations with Leptin Levels

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Abstract

Children with congenital heart disease (CHD) prone to stunted growth by different parameters including weight, height and head circumference compared to a control groups, to accomplish such aim (110) children aged ranged (20 days – 13 years with CHD) have enrolled in this study, (83) patients with a cyanotic CHD (75.45%) and (27) cyanotic type (24.54%) were compared with (171) healthy controls of similar age and sex groups. This study was done in Al-Hilla teaching hospital during the period November 1, 2017 to October 11, 2018. The results indicate a significant (2.06) retardation in all parameters in patients with CHD related to control and there is a significant retardation in weight (z = 3.06) in children with cyanotic CHD related to a cyanotic type. Also there is a significant retardation (z = 1.01) in height gain in children with cyanotic related to a cyanotic type and finally there is a significant (z = 2.09) decrease in head circumference in children with cyanotic CHD related to a cyanotic type. Serum leptin levels were also lowered in all patients with CHD. The results concluded that children with (CHD) experience early and continues decrement of all growth parameter during their life.

Keywords: (children, congenital heart diseases, growth parameters, leptin levels)

Introduction

Congenital heart disease is defined as gross structural abnormality of the heart or intrathoracic great vessels that is actually or potentially of significant¹. It is considered as third congenital diseases in children and a leading cause of death in infant during first year of life². It prevalence is 5 to 8 per 1000 live births that varied in different parts of the world, in a recent study the prevalence has been reported to be ranged 4 to 50 cases per 1000 live Births²,¹⁴,¹⁶.

Several genetic and environmental risk factors have been introduced for CHD, and most important factors include genetic mutation, alcohol drinking, abusing some drugs (most famous thalidomide and cocaine using during pregnancy)⁴,¹⁷. The cytokines in many studies were found to have a strong effect on feeding, weight and energy intake in patients with CHD⁵. Impaired absorption can also be an important cause of malnutrition in CHD, therefore, children with CHD and delayed growth due to increased work of a cardiopulmonary and consequently, fatigue and loss of appetite, dyspnea, tachypnea and chronic hypoxia were directed to malnutrition⁴,⁸,¹⁵,²¹.

Recent studies shows series of serum factors such as leptin, ghrelin and tumor necrosis factor alpha (TNF-) will be changed in these patients. Consequently the rate of absorption of nutrients, growth, weight, energy consumption and storage are changing¹¹. These children with CHD have normal growth when receiving more calories compared to healthy ones⁹,¹² and malnutrition in these patients effects on the metabolic response to injury and complications and outcomes of cardiac surgery including sepsis, renal dysfunction, necrotizing enterocolitis, hospitalization days¹³,²¹.

The CHD is divided into four major groups of cyanotic with and without an increase in pulmonary artery pressure and a cyanotic heart defect with and without an increase in pulmonary artery pressure¹,²⁰. Since it has been shown that the prevalence of growth parameters retardation with CHD were increased in the
last decade in Iraqi population and since leptin hormone has been shown to be involved in long-term regulation of energy balance by suppressing appetite and stimulating weight loss(18) and such correlation has not been studied in Hilla (Babylon province), therefore, this study was undertaken to investigate:

1- The effect of CHD on anthropometries measurements of growth in children involving Weight, height and head circumference between control and cyanotic and a cyanotic CHD.

2- The variation in the effect of cyanotic and a cyanotic CHD on growth parameters.

3- Correlation of serum leptin levels with CHD.

**Materials and Method**

A study of (110) patients with CHD, (83) with a cyanotic CHD and (27) cyanotic CHD and (171) controls was carried out between November 1, 2017 to October 11, 2018 in AL Hilla Teaching Hospital. These patients divided into four groups according to their age. The criteria used for including the patients in the study are:

a) Patients with cyanotic and acyanotic CHD.

b) The age of patients ranged from (20 days – 13 years).

c) Parental consent.

The patients with other congenital anomalies, with chronic disease or acquired heart diseases were excluded from the study. The control groups were selected from general paediatric and neotal care clinics, and no pathological findings had been shown in their clinical examination. The patients and control were subjected to the following:

- Complete history and physical examination.

- Anthropometric measurement including length in cm, weight in kg and head circumference in cm.

- Investigation including:
  1- CXR interpreted by a radiologist
  2- E.C.G. standardized at 25 mm/s
  3- Echo Philips clearvue 350. USA. Used modes: M-mode, 2D and Doppler both continues and colour.

4- Blood samples were collected by venipuncture from all patients and controls. Samples were centrifuged at 3000 rpm for 20 minutes. Clear sera were separated and kept frozen at -20°C until the time of assay. Leptin was measured using the DRG Elisa (DRG international, Inc., USA) by indirect enzyme linked immunosorbent assay according to the protocol provided by manufacturer. The intensity of the colour developed is proportional to the concentration of leptin in the sample, the absorbance is measured at 450 nm and concentration were determine from standard curve.

**Statistical Analysis**

The -Z- Statistical test was done to the significance in difference of cyanotic and a cyanotic CHD on growth parameters in comparison with control (Daniel, 2009) (19).

The level of significance taken 95%

\[ Z_C = \]

If \( Z_C > 1.96 \) mean there is significant differences

**Results**

The hundred ten children with CHD represented by (83), 75.45% acyanotic group compared with cyanotic CHD (27) 24.54%. The patients were classified into four groups according to their age. (table 1).

**Table (1): Classification of children according to age**

<table>
<thead>
<tr>
<th>Age</th>
<th>Control</th>
<th></th>
<th>A cyanotic CHD</th>
<th></th>
<th>Cyanotic CHD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
<td>%</td>
<td>Number</td>
</tr>
<tr>
<td>&lt;1y</td>
<td>29</td>
<td>16.45%</td>
<td>43</td>
<td>51.80%</td>
<td>17</td>
</tr>
<tr>
<td>1-2y</td>
<td>35</td>
<td>20.46%</td>
<td>14</td>
<td>16.86%</td>
<td>5</td>
</tr>
<tr>
<td>2-5y</td>
<td>65</td>
<td>38.01%</td>
<td>14</td>
<td>16.86%</td>
<td>3</td>
</tr>
<tr>
<td>&gt;5y</td>
<td>42</td>
<td>24.56%</td>
<td>12</td>
<td>14.45%</td>
<td>2</td>
</tr>
</tbody>
</table>
Table (2): The effect of CHD on head circumference

<table>
<thead>
<tr>
<th>Centile</th>
<th>&lt;1y</th>
<th>1-2y</th>
<th>2-5y</th>
<th>&gt;5y</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control %</td>
<td>Acyanotic %</td>
<td>Cya- notic %</td>
<td>Control %</td>
</tr>
<tr>
<td>&lt;3rd</td>
<td>-</td>
<td>2.32</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3rd</td>
<td>3.44</td>
<td>30.23</td>
<td>17.64</td>
<td>-</td>
</tr>
<tr>
<td>10th</td>
<td>-</td>
<td>16.27</td>
<td>17.64</td>
<td>2.58</td>
</tr>
<tr>
<td>25th</td>
<td>10.34</td>
<td>2.32</td>
<td>17.64</td>
<td>25.71</td>
</tr>
<tr>
<td>50th</td>
<td>31.01</td>
<td>30.23</td>
<td>23.52</td>
<td>28.57</td>
</tr>
<tr>
<td>75th</td>
<td>37.97</td>
<td>6.97</td>
<td>17.64</td>
<td>25.71</td>
</tr>
<tr>
<td>95th</td>
<td>20.68</td>
<td>9.3</td>
<td>5.88</td>
<td>17.14</td>
</tr>
<tr>
<td>&gt;95th</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Leptin levels were found to be significantly lowered in patients with cyanotic and acyanotic groups compared with controls (P<0.05), Table (3).

Table (3): Serum Leptin Levels in Cyanotic and Acyanotic CHD

<table>
<thead>
<tr>
<th>Acyanotic</th>
<th>cyanotic</th>
<th>control groups</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin (ng/ml)</td>
<td>1.91.4</td>
<td>1.7 2.1</td>
<td>3.7 2.4</td>
</tr>
<tr>
<td></td>
<td>P1 = 0.28</td>
<td>P2 = 0.02</td>
<td>P3 = 0.01</td>
</tr>
</tbody>
</table>

Data were expressed as mean standard deviation.

P1: Acyanotic versus cyanotic groups.
P2: Acyanotic versus control groups.
P3: Cyanotic versus control groups.

Discussion

The study shows a statistical significant (z = 2.06) retardation in all growth parameters involving all age groups (below 1 year, 1-2 years, 2-5 years and above 5 years) but there is no significant (z = 1.2) differences between cyanotic and a cyanotic CHD in relation to any group. Table (1) shows more details, however, there is early presentation and diagnosis for children with cyanotic CHD possibly due to more aggressive clinical presentation in the infancy which attracts the parents attention to seek early medical help.

This study shows significant (z = 3.06) retardation in weight between groups of children with CHD and control, also shows significant (z = 2.04) retardation in children with cyanotic CHD, compared with a cyanotic groups, and this is an expected result due to more harmful effect of CHD (cyanotic) on the nutritional
status of children related to shortness of breath which presented as early failure to gain weight that may lead later on to growth retardation with its complications, (Figure 1) shows that weight retardation below the third centile (100%) in children more than 5 years with cyanotic CHD, also more effect of a cyanotic CHD on both groups (<1 year and 1–2 years, Figure 1) more than other groups (<1 year, 53.48%) at the third centile and more details can be shown on (Figure 1), these findings were consistent with other reports(14), mentioned that children with CHD experience early simultaneous decrease in growth trajectory across weight, length and head circumference, this decrement suggests a role for altered growth retardation in child with CHD.

There is a statistically significant (z = 2.28) retardation in height in children with CHD in comparison with controls, but there is a non-significant (z = 1.01) variation between cyanotic and a cyanotic type. These results were also comparable with finding where (52%) below the 16th centile for both length and weight and 27% were below the 3rd centile for weight and lengths, these comparable results probably indicated that the cause of growth retardation in CHD was multifactorial which could be due to inadequate caloric intake and feeding difficulty(11), in our study there was (25.92%) of cyanotic CHD retardation in height and (42.16%) of a cyanotic type with retardation in height, (Figure 2) shows that 50% of children with cyanotic CHD above 5 years at third centile mostly in cyanotic type.

Head circumference was significantly (z = 2.56) reduced in CHD children in comparison with controls, also there was significant (z = 2.09) retardation in cyanotic type related to acyanotic, this may be explained by early affection of children with cyanotic CHD related to severe symptoms associated with severe growth retardation that lead to affection of skull development later on, (Table 2, Figure 3) shows (50%) of cyanotic CHD children aging above 5 years their head circumference on the 3rd centile comparing with acyanotic type (8.33%) reach 95th centile, also (50%) of cyanotic type below 3rd centile while there is less effect of a cyanotic CHD in early infancy, these results were also agreed with Barbara(3) who mentioned that children with CHD had stunted growth and require feeding supplementation in nearly a quarter of them (during infancy) to meet the definition of failure to thrive in first year of life(3).

Leptin is a hormone that is produced mainly by the fatty tissue and released into peripheral circulation and binds to receptors in the hypothalamus to transmit information about triglyceride content of adipocyte, in addition to macronutrient content and energy composition of newly administered food(10). Low levels of leptin have been found to increase activity of orexigenic peptides and decrease activity of anorexigenic peptides, thereby, increasing appetite and stimulating weight gain(17). Our study demonstrated that serum leptin levels were significantly lowered compared with controls. These results were consistent with a researcher who found that children with CHD had lowered leptin levels than healthy controls(18), while other researcher found no significant difference in plasma leptin levels between cyanotic and acyanotic patients, however, these studies did not include a healthy control groups to compare with(11,18). This study also showed that all the anthropometric parameters in all groups were positively correlated with leptin levels. These findings were supported by others(9,11,12).

This study has some limitation since it was a single center study done in Babylon Province and in addition to hemodynamic variability associated with various types of CHD(11). It was concluded that children with CHD are at increased risk for poor growth parameters and reduced leptin levels in these patients suggesting a role for such hormone in regulating food intake, energy balance and maintenance of body weight. The abnormal hemodynamics and the hypermetabolic state of these patients will compromise nutrition and decreases IGF-1 synthesis with subsequent slowing of linear growth weight gain(20,16).

The incidence of growth disorders in these patients can emphasized that growth retardation in patients with CHD compared with healthy children can be attributed to differences in the factors affecting the growth of CHD children including gender, age, cardiac abnormalities simultaneous multiple valvular lesions and ultimately congestive heart failure that leads to multiple growth problems(3,17).

**Conclusions**

- There was significant decrease in all growth parameters (z = 2.06) in children with CHD related to control.
- There was a significant retardation in weight (z = 2.09) and head circumference (z = 2.09) between cyanotic and a cyanotic CHD.
- There was a non-significant retardation (z = 1.01) in height between cyanotic CHD and a cyanotic CHD.

- Serum leptin levels were lowered in both cyanotic and acyanotic patients with CHD.

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**Conflict of Interest:** The authors declare that they have no conflict of interest.

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**References**


Hormonal and Mineral Imbalance Effect on Bone Resorption in Predialysis Iraqi Patients with Chronic Kidney Disease

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Abstract

Introduction: Chronic kidney disease mineral bone disorder is a metabolic bone disease present in almost all uremic patients. The aim of this research to indicate the stage of chronic kidney disease (CKD) that affect the bone metabolism that leading to the mineral and hormonal imbalance by studying the relationship among osteocalcin, glomerular filtration rate (GFR), parathyroid hormone, calcium and phosphorus levels in the blood.

Method: The study included 52 patients with predialysis chronic kidney disease stage 3-5 and 40 apparently healthy relatives accompanying the patients. Glomerular filtration rate (GFR) was calculated for each patient. Renal function tests, including serum levels of urea, creatinine, a biochemical marker of bone metabolism: osteocalcin (OSN), calcium, phosphorus, and parathyroid hormone (PTH), were measured for each participant.

Results: Serum urea and creatinine levels were significantly higher in CKD patients than that of apparently healthy control. There is significantly higher serum parathyroid hormone, serum phosphorus, serum osteocalcin (P<0.01, P<0.01, P<0.01 respectively) in CKD patients than that of the healthy control group. While low serum calcium level in CKD patients as compared to the corresponding group (P<0.01).

Conclusion: Hyperphosphatemia and hypocalcemia in the end stage of predialysis CKD patients lead to increase parathyroid hormone secretion, which causes high bone turnover characterized by significantly high serum osteocalcin in these patients. Parathyroid hormone and osteocalcin were used as a biomarker for the development of bone and mineral disorders in predialysis CKD patients.

Keywords: CKD, parathyroid hormone, osteocalcin, calcium, phosphorus

Introduction

Chronic kidney disease (CKD) has become a public health problem. The definition of CKD was introduced by National Kidney Foundation in 2002 and later adopted by the international group Kidney Disease Improving Global Outcomes in 2004, a decrease in kidney function with a glomerular filtration rate (GFR) < 60 mL/min per 1.73 m² and/or kidney damage for 3 months or more [1]. Chronic kidney disease, mineral bone disorder (CKD-MBD) is a metabolic bone disease present in almost all uremic patients. With uremia, bone is relatively resistant to parathyroid hormone (PTH) action, such that the average level of PTH is required to maintain bone turnover [2]. Relative hypoparathyroidism is associated with low-turnover or a dynamic bone disease [3]; severe secondary hyperparathyroidism leads to high turnover bone disease [4]. Secondary hyperparathyroidism occurs in CKD produces an imbalance between osteoclast activity and osteoblast synthetic activity, leading to enhanced bone breakdown at the end stage of renal disease [5].

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Osteocalcin (OSN) is a non-collagenous, vitamin K-dependent protein with 46–50 amino acid, produced by osteoblasts, it is a marker of bone formation [6]. High blood OSN levels are also in older adult humans, high levels in the blood are a predictor of lower bone density and a sign of fracture risk, including hip fractures and this because OSN levels can increase in the blood as a result of the breakdown of bone tissue [7]. The circulated OSN is removed by the kidney and liver [8], which increase in the blood when the renal function is declining. Patients with CKD show a progressive increase in serum OSN levels that closely corresponded with intact PTH and alkaline phosphatase levels. More fundamentally, such increases in serum OSN levels reflect the severity of the bone lesions [9].

Small increases in serum OSN were found in some patients at a severe stage, while a significant increase in blood OSN is shown in patients with end stage predialysis. In such patients, this elevation either due to decreased renal clearance of OSN or also reflected increased bone metabolism [10, 11]. There is a negative correlation between glomerular filtration rate (GFR) and plasma osteocalcin levels in predialysis patients [12].

The aim of this research to indicate the stage of chronic kidney disease that affects the bone metabolism that leading to the mineral and hormonal imbalance by studying the relationship among OSN, GFR, PTH, calcium and phosphorus levels in the blood.

Method

The case-control study was conducted from March to Jun 2019, at the National Center of Teaching Laboratories of Medical City Institute, Baghdad, Iraq. Data of predialysis CKD Iraqi patient’s attendant to Ghazi Alhariry hospital in Medical City in March 2019 for renal evaluation function were included in the study. The study was approved by the Ethics Committee of the University of Baghdad, Faculty of Pharmacy (UBCP-RECA-M62019A). 52 patients with predialysis chronic kidney disease stage 3-5 were enrolled, and 40 apparently healthy relatives accompanying the patients were selected. The purpose of the study and nature of all procedures were explained to participants, and informed approval was obtained before the commencement of the study. Patients were excluded if they had an acute infection, cancer, acute myocardial infarction, pulmonary edema, and patients on medication (steroid, bisphosphonates, calcium or vitamin D). The diagnosis of predialysis CKD patients was made by nephrologist based on the estimation of GFR together with renal function tests [table1]. GFR was calculated by the Modification of Diet in Renal Disease (MDRD) equation: 186 x (Creatinine/88.4)+1.154 x (Age)-0.203 x (0.742 if female) x (1.210 if black). Moderate reduction of GFR (30–59 mL/min/1.73 m²) in stage 3, severe reduction of GFR (15–29 mL/min/1.73 m²) in stage 4 preparation for renal replacement therapy and established kidney failure or end-stage renal disease (ESRD) (GFR <15 mL/min/1.73 m²) in stage 5 requiring permanent renal replacement therapy (RRT).

The serum OSN was determined by Chemiluminescent enzyme immunoassay using Immulite 1000 autoanalyzer [13] (LKON1Siemens, USA). Serum urea nitrogen was measured by using urease/glutamate dehydrogenase coupled enzymatic technique (Dimension clinical chemistry System, DF21 Siemens, USA). Serum creatinine was measured by using modified kinetic Jaffe technique (Dimension clinical chemistry System, DF33B Siemens, USA). Serum intact PTH was measured by using two-site chemiluminescent enzyme-labeled immunometric assay [14] (DPC Immulite 2000, Siemens, USA). Serum calcium and phosphorus levels were quantified by using Ca(DF23A), PO₄ (DF61A) Dimension Rxl Siemens autoanalyzer as per International Federation of Clinical Chemistry (IFCC) guidelines, modifications of calcium O-cresolphthalein complex one reaction (OCPC) and classical phosphomolybdate method, respectively [15, 16].

Statistical Analysis

It was performed by using the SPSS Statistics version 20.0 The results were expressed as mean and standard deviation. Results were analyzed utilizing One-way ANOVA was used to determine the significance degree between parameters. The p-value ≤0.05 was considered significant. ROC curve was used to identify the validity of markers as an indicator of the disease.

Results

Serum levels of both urea and creatinine levels were significantly higher in CKD patients than apparently healthy control. These results were used together with the estimated GFR (< 60 mL/min per 1.73 m²) to determine the stage of CKD in patients group [table 1].
Table 1: Descriptive statistics between stages of CKD patients and healthy control.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Stages</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>ANOVA test (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age / Year</td>
<td>A.H. Control</td>
<td>40</td>
<td>44.61</td>
<td>11.505</td>
<td>P=0.00*</td>
</tr>
<tr>
<td></td>
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<td>PTH</td>
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<td>Total</td>
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</tbody>
</table>
*A.H : apparently healthy, CKD: chronic kidney disease, GFR: glomerular filtration rate, OSN: osteocalcin, PTH: parathyroid hormone Statistically significant at p <0.05, statistically highly significant at p-value ≤0.01 and non-significant at p>0.05

Curves of ROC studies confirmed that the blood urea and serum creatinine were a highly sensitive and specific diagnostic marker of CKD [figure1]. The sensitivity, specificity and cut-off point of hormonal and minerals for predialysis patients with CKD were estimated by ROC [table 2]

Figure 1: ROC for serum urea and creatinine levels

Figure 2: ROC curves for PTH, OSN, serum Calcium and serum phosphorus
Table 2: The cut-off point with sensitivity 100, specificity 100 and Area under the curve (AUC) 1.000 of laboratory results

<table>
<thead>
<tr>
<th>Validity tests</th>
<th>Serum urea</th>
<th>Serum Creatinine</th>
<th>Serum Calcium</th>
<th>Serum phosphorous</th>
<th>OSN</th>
<th>PTH</th>
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<tr>
<td>Sensitivity</td>
<td>100%</td>
<td>100%</td>
<td>80%</td>
<td>86.7%</td>
<td>91.1%</td>
<td>95.6%</td>
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<tr>
<td>Specificity</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>61.1%</td>
<td>77.8%</td>
<td>50%</td>
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<tr>
<td>Area Under the curve (AUC)</td>
<td>1</td>
<td>1</td>
<td>0.972</td>
<td>0.881</td>
<td>0.928</td>
<td>0.957</td>
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<tr>
<td>Cutoff value</td>
<td>&gt; 38</td>
<td>&gt; 1.2</td>
<td>&lt; 8.5</td>
<td>&gt; 3.1</td>
<td>&gt; 6.6</td>
<td>&gt; 21.5</td>
</tr>
<tr>
<td>P-value</td>
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<td>0.00 HS</td>
<td>0.00 HS</td>
<td>0.00 HS</td>
<td>0.00 HS</td>
<td>0.00 HS</td>
</tr>
</tbody>
</table>

HS=Highly significant difference (P<0.01)

Discussion

In the present study, the end stage of CKD patients has significantly higher serum concentration of the urea and creatinine as compared to stages 3-4 of CKD patients which is in agreement with other study[17]. This is due to the progressive reduction of GFR at the end stage of CKD patients. As the GFR decreases blood levels of both urea and creatinine are increased [18]. Significantly higher serum PTH levels at the end stage of CKD group among all studied patients groups, which is agree with the other findings which reported that the serum PTH was significantly higher in more advanced renal failure (stage 5 CKD), which confirms the relationship between severity of hyperparathyroidism and the degree of renal impairment [19,20]. Secondary hyperparathyroidism result from a decreased renal function, which is a common complication of CKD that leads to an overproduction of PTH caused by several changes that occur in bone and mineral metabolism because of decreased kidney function [21]. In this study, the CKD patients have hyperphosphatemia and hypocalcemia that leads to significant hyperparathyroidism when compared to healthy control patients with normal renal function.

At the end stage of CKD, when the remaining nephrons can no longer sufficiently excrete the phosphorus load, hyperphosphatemia is detected. The calcium and phosphorus form an insoluble complex in serum. This process may lead to extraskeletal calcification and potentially calciphylaxis or cardiac disease [22]. Retention of phosphorus also indirectly causes excessive production and secretion of PTH through lowering of ionized Ca\(^{2+}\) and by suppression of calcitriol production [19]. Vikrant S et al. was found increase serum PTH level in the end stage of CKD inversely correlated with GFR and serum calcium and positive correlation with serum phosphorus which is in agreement with this study [23]. Hyperphosphatemia is recognized as the primary initiator of the various cascades of the promoters of renal bone disease [24].

The present study shows increment serum osteocalcin levels associated with progressing stage of CKD up to higher concentration in the end stage CKD. Rix et al. who reported that patients with predialysis CKD had elevated serum levels of OSN with the more severe stage of CKD corresponding to the level of secondary hyperparathyroidism [25] as in agreement with this study.

In patients with impaired renal function plasma OSN levels are markedly elevated due to increased bone turnover and decreased renal elimination [26]. The effects of increased parathyroid hormone (PTH) resultant...
resistance to adaptive stimulation of bone formation by parathyroid hormone, permits the effects of kidney injury to inhibit bone formation despite the development of secondary hyperparathyroidism [27].

Phosphate retention, hypocalcemia, and bony resistance to the action of PTH all these factors may contribute to overactivity of parathyroid gland to increase synthesis and secretion of PTH in end stage CKD. High levels of OSN in blood at the end stage of CKD occurs due to elevated PTH which stimulate bone demineralization that characterized by accelerated rates of bone absorption and resorption.

Conclusion

Hyperphosphatemia and hypocalcemia in the end stage of predialysis CKD patients lead to increase parathyroid hormone secretion, which causes high bone turnover characterized by significantly high serum osteocalcin in these patients. PTH and OSN were used as a biomarker for the development of bone and mineral disorders in predialysis CKD patients. It is recommended that attending physicians monitor and control biochemical parameters early in the development of CKD before the need for dialysis to protect the CKD patients from any complications that will result in response to PTH.

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Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

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13. Fassbender WJ, Steinhauer B, Stracke H, Schumm-


Robust Controller Electromyogram Prosthetic Hand with Artificial Neural Network Control and Position

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¹College of Engineering- University of Gaziantep-Turkey, ²Technical College of Kirkuk- Northern Technical University-Iraq, ³College of Engineering -University of Abdullah Gül, Turkey

Abstract

In this study, we proposed and designed a new control method for an electromyographically (EMG) controlled prosthetic hand. The objective is to increase the control efficiency of the human–machine interface and afford greater control of the prosthetic hand. The process works as follows: EMG biomedical signals acquired from Myoware sensors positioned on the relevant muscles are sent to the robot that consist of hand, Arduino and MATLAB program, which computes and controls the hand position in free space along with hand grasping operations. The Myoware device acquires muscle signals and sends them to the Arduino. The Arduino analyzes the received signals, based on which it controls the motor movement. In this design, the muscle signals are read and saved in a MATLAB system file. After program processing on the industrial hand which is applied by MATLAB simulation, the corresponding movement is transferred to the hand, enabling movements, such as, hand opening and closing according to the signal stored in the MATLAB system. In this study, hand and fingerprints were designed using a three-dimensional printer by separate recording finger and thumb signals. The muscle signals were then analyzed in order to obtain peak signal points and convert them into data. These results indicate the effectiveness of the proposed method and demonstrate the superiority of the method for amputees because of the improved controllability and perceptibility afforded by the design.

Keywords: Arduino controller., Electromyography, Hand robot, Prosthetic hand.

Introduction

Nowadays, the development of science and technology has led to prosthetic devices with promising functional capabilities and esthetic appearance in research domain in favor of commercialization¹.

The design of prosthetic hand is multidisciplinary, compelling knowledge of physiology, anatomy, electrical and electronics, mechanical design, software, and so on, depending on the nature of control Robotic prosthetic hands have attracted considerable attention in terms of their practical use by amputees. Artificial neural network is used to classify the signal features and subsequently recognize the performed movement ². Along this research line, Sumit et al. conducted real-time identification of active hand-movement EMG signals based on wrist-hand mobility for simultaneous control of prosthetic robotic hands ³. In another system, a fully wireless, mobile platform used for acquisition and communication of sEMG signals is embedded in a mobile control system, and Ottobock 13E200 EMG electrodes are used to acquire the EMG signals. The electrodes are attached to the patient’s remaining forearm stump. In addition, a laptop is used to provide the required computational power for the control of the prosthetic robotic hand ⁴. In the light of reducing costs, some studies have utilized an open-source design for the implementation of affordable, modular, compliant, under-actuated prosthetic fingers that can aid amputees who suffer from partial amputations (e.g., amputations of one or several fingers of the human hand, with the exception of the thumb) to regain lost dexterity ⁵. In general, the control design of a robotic arm employs fuzzy algorithms to interpret EMG signals from the flexor carpi radialis, extensor carpi radialis, and biceps brachii.
muscles. In one type of control approach, the control and acquisition system consists of a microprocessor, analog filtering, digital filtering and frequency analysis, and a fuzzy control system, and electromyographic grasp recognition together with an 8-bit microcontroller is used to control a veneered robotic hand to emulate six grasp types that are used for over 70% of daily activities. A new configuration of sEMG electrodes has been reported to reduce interference resulting from electrode shift depending on muscles movement. The authors suggested that optimizing electrode configuration can improve the EMG pattern discrimination, wherein the proposed electrode configuration has a reference value.

Myoelectric prosthetic hands are primarily intended for adults but are also made by many companies for commercial purposes as prosthetic hand for children. It is also noteworthy that almost all robotic hands designed in university research projects consist of numerous actuators and sensors, which makes them unsuitable for manufacturing along with being too expensive for the typical user. In general, the medical industry can greatly benefit from providing low-cost portable systems that allow visualization of patient data easily and remotely while also providing quick access to accurate data in real time, thereby enhancing the efficiency of doctors and specialists along with providing the patient with greater ability and care.

Today, robotics considered as one of the best technologies that deal with design, working and applications of robots, computer systems their control and information processing. These technologies help physiotherapists and robotics engineers to model and design robotic hands. We aim something more than material and physical. As a result, we want to create opportunities for people with no hands and ability to last their daily lives.

**Aim and Objectives**

In this study, hand and finger prints were designed using a three-dimensional printer by separate recording finger and thumb signals. The muscle signals were then analyzed in order to obtain peak signal points and convert them into data. These data are classified according to muscular positions and used for hand control.

**Materials and Method**

**Mechanical Hand Design**

The prototype hand used in the study was a 3D printed version of the Flexy Hand. Therefore, the corresponding STL file was exported into the Makerbot platform and directly printed without any scaling or modification. Along with the separate parts of the hand, the printing of the entire hand took about 11 hours to be completed. The completed hand was strung with a fishing line and a stretched disposable pipette. The disposable pipette was used to clear any excess material from the 3D printing that would hinder the fishing line’s path through the interior of the hand and fingers, and to aid in threading the line through the palm of the hand. Each finger was strung with about two feet of fishing line to ensure that there would be sufficient material to reach down the length of the arm and attach to the servos.

**Motor Control**

A servo motor with three wires, power wire, ground wire, and pulse-width modulation (PWM) wire, was used to drive the hand. The PWM wire was connected to one of the six PWM ports of an Arduino UNO board. The power and ground wires of each servo were connected to the horizontal positive and negative rows on the breadboard that was connected to a 6-V battery pack. The battery pack housed four 1.5-V D-size batteries. The PC module was plugged into the USB cable.

**Hand Control**

In this section, we used an Arduino UNO unit in this study to analyze the EMG signals acquired from the muscles. The signal is handled by the motor, and the UNO board is also used to send PWM information to the motor for hand control. By Using Myo arm band to provide the signal for MATLAB for recording it and simulating the signal after passing from equalization then the command which has been created by MATLAB will be pass to the microcontroller which has the all control to the hand by using servos.

Another benefit of having a simulation of the hand model is that it allows representing the results as functions of parameters (such as, the weight or type of material) to work on further improvements. Lastly, the grasp quality and optimization of the finger positions for different grasps are other crucial aspects that can be tested with a good model. The Simulink program is designed for multidomain simulation and model-based design. As mentioned previously, Simulink has the ability to simulate and generate automatic code, and allows conducting various tests along with verification.
of the embedded systems. Further, Simulink enables users to incorporate their MATLAB algorithms into models and export the simulation results to MATLAB for additional analysis.. The Simulink program consists of the following commands: constant, Slider Gain, (Sum, Add, Subtract, and Sum of Elements), Sine Wave, and Arduino IO servo Write. The constant is used for generating a real or complex constant value. The Slider Gain is used for varying the scalar gain during the simulation by using the slider; this block has one input and one output. The sine wave is used for generating a sinusoidal waveform, thus indicating that the output of this block is sinusoidal. The sine wave and Slider Gain signals are directed to the mixer, whose output is the summation of the sine wave and Slider Gain signals. The output of the mixer forms the input of the servo Write.

**EMG Signals**

In this study, we used an EMG shield to obtain the signals from the arm muscles, and because the signals were not very clear, we used another high-sensitivity device to obtain clear signals.

\[ y = \frac{1}{N} \sum_{i=1}^{N} |X_i| \]  .......................................(1)

Where \( N \) is the length of the signal and \( X_k \) represents the EMG signal in a segment. A simple way to measure the level of muscle activity is absolute value and this feature is common for use in myoelectric control. This feature is used for all classification in this project.

Root mean square:

\[ y = \frac{1}{N} \sqrt{\sum_{i=1}^{N} X_i^2} \]  .......................................(2)

Slope sign change:

\[ y = \sum_{i=1}^{N-1} (|X_{i+1} - X_i|) \]  .......................................(4)

Zero crossings:

\[ y = \sum_{i}^{N-1} f(X) \]

\[ y = \sum_{i}^{N-1} f(|X_{i+1} - X_i|) \]

**Cost Analysis**

Table 1 lists the cost breakdown of the entire system, including the price of 3D printing. The prices of electronic components (resistors, capacitors, wires, solder), and mechanical component (screws, nuts, bolts, crimps, silicone) are not listed since they can be acquired easily from campus laboratories and machine shop. The cost of the project stayed well within the estimated limit. Compared to the existing advanced robotic hands that are available on the market, which cost around 90,000 $, a 350 $ robotic hand solution seems more viable to the general population of users.

**Results and Discussion**

In this section, we compared the performances of our device and other existing devices in the market. Here, we remark that while our hand design is based on the ideas underlying the normally manufactured industrial robotic hand, we have also added a servo motor and EMG-based grip control in order to improve the device performance as well as ensure weight reduction relative to the weights of previously manufactured hands. The full weight of our device is 500 g, and the cost is as low as $250. These benefits are possible due to the use of EMG and grip control. The table 2 compares our device
with certain other devices in the market. Comparison parameters include type, weight, and grip pattern along with other key parameters. From the table, it is obvious that our device is more feasible for practical application than other existing devices. In terms of device weight, our hand lies in the weight range of the myoelectrically controlled powered hand prosthetic and the BeBionic (RSL Stepper), whose weights are considered suitable for prosthetic hands. Overall, our findings indicate the proposed robotic hand delivers a satisfactory performance, particularly in terms of improved controllability and perceptibility over other devices. An added benefit is the fact our device is less expensive than other devices.

Due to an experimental limitation and difficulties problems or complications with reliably dependably performing each every gesture, different users had the latency check was solely done on one user. Every gesture was performed five times from an amount of rest and control till the motion completion time may well be determined resolute whereas the myogram activity and sophistication labels tags were unendingly recorded. Table 3 shows the results of testing the hand on 7 persons who were missing an arm. The data set is for two movements; namely, the closing and opening of the hand. Some of the results have different rates of errors due to errors beyond the control of the designed system. One of common reason are the differences in the human muscles. In general, with regard to the design of robotic prostheses, the primary challenge involves developing a flexible experimental setup for closed loop control of a prosthetic device with integrated augmented reality that allows changing the extent and type of provided visual and vibrotactile feedback. Several studies have focused on the control approach as well. In one study, thirteen volunteers participated in the experiments by controlling the Ottobock Sensor Hand Speed prosthesis 12. The results indicated that the recorded vibrotactile patterns were able to replace visual feedback. In another study, in a multi-sensory, five-fingered Bio-Mechatronic hand with an sEMG interface, each finger was integrated with torque and position sensors that offered the hand more grasping patterns and complex control methods13. Arduino code could be uploaded to an online repository and made freely available for download. This code would incorporate controller programming, and a Graphical UI (GUI) that would enable the client to tune the control calculation to their inclination. The client would then have the option to purchase modest diversion gadgets, transfer the product themselves to the controller, and introduce the controller into the Talonhand themselves14.

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Table 2. Comparison of existing commercial myoelectric prosthetics and our design

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<th>Name</th>
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<th>Grip pattern</th>
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<th>Grip control</th>
<th>Cosmetic cover</th>
<th>Adaptive grip</th>
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<td>500 g</td>
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<td>1</td>
<td>EMG</td>
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<td>No</td>
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<td>BeBionic (RSL Steeper)</td>
<td>sEMG</td>
<td>550 g</td>
<td>14</td>
<td>1</td>
<td>Smart-phone app</td>
<td>Yes</td>
<td>Yes</td>
<td>25000-35000 USD</td>
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<tr>
<td>i-Limb (Touch Bionics)</td>
<td>sEMG</td>
<td>460 g</td>
<td>14</td>
<td>1</td>
<td>Smart-phone app</td>
<td>Yes</td>
<td>Yes</td>
<td>20000-100000 USD</td>
</tr>
<tr>
<td>Deka Arm (DEKA)</td>
<td>TRI</td>
<td>-</td>
<td>6</td>
<td>4</td>
<td>EMG</td>
<td>No</td>
<td>No</td>
<td>100000 USD</td>
</tr>
<tr>
<td>Michelangelo (Ottobock)</td>
<td>sEMG</td>
<td>420 g</td>
<td>6</td>
<td>1</td>
<td>Remote control</td>
<td>Yes</td>
<td>No</td>
<td>100000 USD</td>
</tr>
</tbody>
</table>

Table 3. Results of testing the hand on 7 persons

<table>
<thead>
<tr>
<th>Motion Gesture</th>
<th>PT1</th>
<th>PT2</th>
<th>PT3</th>
<th>PT4</th>
<th>PT5</th>
<th>PT6</th>
<th>PT7</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open</td>
<td>80/100</td>
<td>90/100</td>
<td>85/100</td>
<td>80/100</td>
<td>95/100</td>
<td>75/100</td>
<td>90/100</td>
<td>85/100</td>
</tr>
<tr>
<td>Close</td>
<td>83/100</td>
<td>85/100</td>
<td>91/100</td>
<td>80/100</td>
<td>90/100</td>
<td>70/10</td>
<td>85/100</td>
<td>83/100</td>
</tr>
</tbody>
</table>

PT: Patients

**Conclusion**

From the results of this study, it is concluded that a simple and cost-effective prosthetic hand can significantly contribute to the development of robotic prosthesis and the results indicate the effectiveness of the proposed method and demonstrate the superiority of the method for amputees because of the improved controllability and perceptibility afforded by the design.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** Self-funding

**References**


12- Ninu, A., Dosen, S., Muceli, S., Rattay, F., Dietl, H., & Farina, Closed-loop control of grasping with a myoelectric hand prosthesis: Which are the relevant feedback variables for force control. 2014 IEEE transactions on neural systems and rehabilitation engineering. 22(5), 1041-1052.


Study the Genotoxicity of Aqueous and Alcoholic Extracts of *Adhatoda Vasica* on the Roots of *Allium cepa* L. by RAPD-PCR Technique

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¹Iraq/University of Baghdad – College of Education /Ibn-Al-Haitham-Biology dept.

Abstract

The Molecular technique RAPD-PCR used to detect the genotoxicity of different concentrations of aqueous and alcoholic extracts of *Adhatoda vasica* leaves on onion *Allium cepa* L. roots. Five concentrations (1%, 2%, 3%, 5%, and 7.5%) and (10%, 15%, 20%, 30%, and 40%) were adopted for alcoholic and aqueous extracts respectively. Ten arbitrary primers used in this study, only eight showed polymorphic bands in the gel and two primers were neglected because they did not show any polymorphic bands for all samples. The aim of this study is to detect the toxic effect of the extracts on the root of onion. The emergence and disappearance of bundles in the genome of onion plant *Allium cepa* L. Treatment with extracts was studied. The genetic relationship tree was established and the genetic distance calculated based on the results obtained from the gel electrophoresis to clarify the toxic effects of the extracts. The results showed that the concentrations that gave the highest effect and the most toxic for aqueous extract is 40% and for the alcoholic extract is 7.5% which are recommended as effective concentrates if used as a pesticide.

**Key words:** RAPD, *Adhatoda vasica*, Extracts, genotoxicity, *Allium cepa* L.

Introduction

Acanthaceae a family that composed of many well-known medicinal plants comprises approximately of 3,400 species and 364 genera. The name *Adhatoda vasica* Nees, whose synonym is *Justucia adhatoda* L.¹ was selected for experiments on its extracts in this study. *A. vasica* is an evergreen plant. It grows in the form of herbaceous trees distributed all over the world and in various environmental conditions, it lives at an altitude of 1300 m above sea level in the Indian Himalayas²,³.

*A. vasica* leaves contain a number of chemicals such as alkaloids, flavonoids, turines, and saponins⁴. Alkaloids as Vasicinine. Vasicinol. Adhatodin. Adhatonine. Antisotine and pegainine⁵. The leaves contain two major alkaloids vasicine and vasicinone⁶. Vasicine alkaloid is one of the most effective substances that can be obtained from the leaves of the plant of the seven throat and by 95% of the isolated alkaloids⁷. The leaves and flowers of *A. vasica* are used as a medicine because they possess many chemical components such as carbohydrates, protein, phenolsas well as flavanoids and alkaloids⁸. *A. vasica* contains saponins and tannins too⁹, and the roots contain many alkaloids as vasicol, vasicinolone, vasicinone, adhatonine, vasicine and vasicinol¹⁰. Medicinal herbs are a very important source of medicine throughout human history. *A. vasica* is a plant known in ancient Greek medicine as a medicinal plant and used in the medical system for more than 2000 years¹¹, and it is widely used today, indicating that herbs are an increasingly important part of modern medicine. About 25 - 30 percent of the prescribed drugs today contain chemicals derived from plants¹². *A. vasica*, with its various parts of high potential plants, is used for the development of the pharmaceutical and drug industries¹³. The plant was used as an anti-asthma and bronchodilator and in the treatment of wounds, ulcers, allergies and pulmonary tuberculosis. Genotoxicity is the sum of DNA damage that causes mutations. When medicinal plants are used to treat diseases, genetic toxicity must be detected. Genotoxicity of *A. vasica* plant extracts. In this study, genotoxicity was detected using the DNA of the onion *Allium cepa* L. roots as a biomarker to confirm the genotoxicity of *A. vasica* extracts.
Materials and Method

Plant sampling

*A. vasica* leaves were collected during the month of September 2018 from the gardens of the University of Baghdad / Baghdad-Iraq, leaf lengths were 12-27 cm and classified by the herbarium of the college of agriculture - University of Baghdad.

Preparation of extracts

Aqueous extract prepared from 25 g of *A. vasica* leaf powder added to 125 mL of distilled water (in boiling degree) so that the solution is easy to filtrate. Then put the solution on the hotplate stirrer for two hours and filter the solution through 4 pieces of gauze, and centrifuged at 3000 rpm. Place the filtrate in Petri dishes and enter the oven at 40 °C. Scrape the extracted leaves after drying and store at room temperature until use (14).

To prepare the alcoholic extract, 125 ml of ethyl alcohol at 70% concentration added to 25 grams of dry powdered leaves and transfer the solution in a 50 °C for 24 hours in the shaking water bath. The steps were followed as in the preparation of aqueous extract (15).

Selection of samples and concentrations of extracts

Onions were selected in medium sizes (1.5, 2.2 cm) and 30 g weight, growing well at root lengths (2.5, 3.5 cm) the old roots were removed with dissecting blade and then the onion bulbs transferred to appropriate sized test tubes containing distilled water for 24 hours, then transferred to bottles containing a series of needed concentrations of alcoholic and aqueous extract of *A. vasica* leaves, adopted five concentrations (1%, 2%, 3%, 5%, 7.5%) and (10%, 15%, 20%, 30%, 40%), for alcoholic and aqueous extracts respectively, the onion bulbs still in their concentrations each for 7 days with considering the change of each solution every 24 hours to avoid increasing of concentration by water evaporation. After expiry of the exposure period, roots removed for further studies (16).

DNA extraction

DNA was extracted from onion roots that grown in each concentration solution studied by CTAB (Cetyl Trimethyl Ammonium Bromide) method (17, 18, 19) following the steps recommended by researchers in those references with some modifications.

Measuring the concentration and purity of DNA

The purity and concentration of DNA were measured using the Nano drop spectrophotometer by placing 2μL of each sample in a designated place of the device. The results appeared in private computer software. The DNA purity ranged from (1.7, 1.9) with different DNA concentrations measured in ng/μl. The quality of the extracted DNA was determined by electrophores the samples onto 1% agarose gel.

PCR-RAPD technique

Primers used in RAPD-PCR reaction shown in Table (1) arbitrary primers supplied by Interganti DNA Technology (IDT,USA), and Polymerase chain reaction PCR program shown in Table (2):

<p>| Table (1) Primers used in this study |</p>
<table>
<thead>
<tr>
<th>No.</th>
<th>Primer</th>
<th>Primer sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OPA-1</td>
<td>5́-CAGGCCCTTC-3́</td>
</tr>
<tr>
<td>2</td>
<td>OPA-2</td>
<td>5́-TGCCGAGCTG-3́</td>
</tr>
<tr>
<td>3</td>
<td>OPA-3</td>
<td>5́-AGTCAGCCAC-3́</td>
</tr>
<tr>
<td>4</td>
<td>OPA-4</td>
<td>5́-AATCGGCGTC-3́</td>
</tr>
<tr>
<td>5</td>
<td>OPA-5</td>
<td>5́-AGGGGTCTTG-3́</td>
</tr>
<tr>
<td>6</td>
<td>OPA-6</td>
<td>5́-GCTCCCTGAC-3́</td>
</tr>
<tr>
<td>7</td>
<td>OPA-7</td>
<td>5́-GAAACGGGTG-3́</td>
</tr>
<tr>
<td>8</td>
<td>OPA-8</td>
<td>5́-GTGACGTAGG-3́</td>
</tr>
<tr>
<td>9</td>
<td>OPA-9</td>
<td>5́-GGGTAACGCC-3́</td>
</tr>
<tr>
<td>10</td>
<td>OPA10</td>
<td>5́-GTGATCAGCAG-3́</td>
</tr>
</tbody>
</table>

<p>| Table (2): PCR program |</p>
<table>
<thead>
<tr>
<th>Cycles No.</th>
<th>Temp. C°</th>
<th>Time</th>
<th>Steps</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>95</td>
<td>4min.</td>
<td>Initial denatur-ation</td>
</tr>
<tr>
<td>40</td>
<td>94</td>
<td>30sec.</td>
<td>Denaturation</td>
</tr>
<tr>
<td></td>
<td>32-40</td>
<td>1min.</td>
<td>Annealing</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>1min.</td>
<td>Extension</td>
</tr>
<tr>
<td>1</td>
<td>72</td>
<td>10</td>
<td>Final extension</td>
</tr>
</tbody>
</table>
Results and Discussion

RAPD –PCR technique

RAPD-PCR technique can be used effectively to detect DNA damage, therefore be used in genotoxicity studies (21, 22). It can be used without prior knowledge about the genome (23). It uses random primers with DNA template sequences (24). It has the ability to detect DNA damage and verify mutations in the DNA when toxic substances are used (25). Using RAPD-PCR technique in this study revealed the genotoxicity of A. vasica leaf extracts as shown in Figure 1. The emergence or acquisition of bands in gel electrophoresis occurred as a result of changes in the structure of the DNA (fracture, transfers or deletions). As for the deletion or loss of bands, it was due to the reduction of the number of areas of binding with the polymerase (25) or mutation or damage at the site of primer binding to the DNA strand, as well as that loss is as a result of DNA damage, point mutation, chromosomal rearrangement, deletion and addition to the sequences of nitrogenous bases in the DNA strands due to genotoxicity effect (26). The primers OPA3, OPA7 were neglected because they did not show any polymorphism in gel bands for all samples.

After determining the purity and concentration of the extracted DNA of the extracts exposed roots, a high concentration of 800 ng/μl was found. Concentrations were reduced to 150 ng/μl for each extract. The results of the RAPD technique showed a variation in the PCR results of samples that exposed to aqueous and alcoholic extracts compared with the control treatment. Table 3 and 4 and Figure 1, show the RAPD-PCR results. It is clear from the results that the eight primers used gave fifty-five bands in control treatment and the molecular weights were between (140-2109). As for the number of lost and gained bands, it was found that the alcoholic extract was more toxic to the onion roots genome than the aqueous extract.

Table (3) Lost and gained gel bands after PCR in roots of onion plant treated with aqueous extract concentrations.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Bands control</th>
<th>Band case</th>
<th>10%</th>
<th>15%</th>
<th>20%</th>
<th>30%</th>
<th>40%</th>
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</thead>
<tbody>
<tr>
<td>OPA-1</td>
<td>7</td>
<td>Gain</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Loss</td>
<td>5</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>OPA-2</td>
<td>11</td>
<td>Gain</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Loss</td>
<td>6</td>
<td>8</td>
<td>6</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>OPA-4</td>
<td>6</td>
<td>Gain</td>
<td>2</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Loss</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>OPA-5</td>
<td>6</td>
<td>Gain</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Loss</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>OPA-6</td>
<td>6</td>
<td>Gain</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Loss</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>OPA-8</td>
<td>7</td>
<td>Gain</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Loss</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>OPA-9</td>
<td>4</td>
<td>Gain</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
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<td>Loss</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>OPA-10</td>
<td>8</td>
<td>Gain</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Loss</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
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<td>53</td>
<td>52</td>
<td>45</td>
<td>37</td>
<td>38</td>
</tr>
</tbody>
</table>
Table (4) Lost and gained gel bands after PCR in roots of onion plant treated with alcoholic extract concentrations.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Bands control</th>
<th>Band case</th>
<th>1%</th>
<th>2%</th>
<th>3%</th>
<th>5%</th>
<th>7.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPA-1</td>
<td>7</td>
<td>Gain</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Loss</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>OPA-2</td>
<td>11</td>
<td>Gain</td>
<td>1</td>
<td>5</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Loss</td>
<td>2</td>
<td>7</td>
<td>6</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>OPA-4</td>
<td>6</td>
<td>Gain</td>
<td>11</td>
<td>7</td>
<td>10</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Loss</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>OPA-5</td>
<td>6</td>
<td>Gain</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
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<td>Loss</td>
<td>3</td>
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<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>OPA-6</td>
<td>6</td>
<td>Gain</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td></td>
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<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>OPA-8</td>
<td>7</td>
<td>Gain</td>
<td>4</td>
<td>6</td>
<td>6</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Loss</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>OPA-9</td>
<td>4</td>
<td>Gain</td>
<td>5</td>
<td>4</td>
<td>6</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Loss</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>FOPA-10</td>
<td>8</td>
<td>Gain</td>
<td>4</td>
<td>2</td>
<td>3</td>
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<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>-</td>
<td>53</td>
<td>59</td>
<td>57</td>
<td>50</td>
<td>39</td>
</tr>
</tbody>
</table>

The present study has shown a decrease in viability to death of the roots of *Allium cepa* when treated with different concentrations of both extracts, suggesting that it can be used as a pesticide to weed. Our findings evoke that Low concentrations were cytotoxic to onion roots while high concentrations of 40% and 7.5% of aqueous and alcoholic extracts respectively were prevented cell entry into mitosis. So it was more harmful to the food chain receptors. Consequently, the plant is unable to absorb it for this reason it is recommended to use low concentrations of both extracts as a pesticide (27).

**Cluster analysis**

UPGMA analysis was used to map the genetic relationship tree (28,29,30) using Jaccard’s similarity coefficients the tree results showed groups below (Figure 2).

Group A contains two groups, A1 group included control sample and onion sample exposed to alcoholic extract at 7.5% control (distilled water) and aqueous extract concentrations 20%, 30%, 40%. Group A2 included onion samples exposed to the aqueous extract concentrations of 10% and 15%.

Group B comprised two groups. Group B1 onion samples exposed to the concentration of alcoholic extract 1%, 2%. Either group B2 included samples exposed to alcoholic extract concentrate 3%, 5%.

The results of the genetic relationship tree showed the isolation of the results of aqueous extract from the alcoholic extract, and the isolation of the results of the exposure to control sample and the alcohol concentration of 7.5% were observed clearly.
The tree showed the isolation of the control sample and the concentration of 7.5% alcohol compared to other samples treated with the extract (aqueous and alcoholic). This indicates that all the concentrations had an effect on the onion genome.

Conclusions

This study revealed that the concentration that gave the highest effect and the most toxic for aqueous extract is 40% and for the alcoholic extract is 7.5% because with these concentrations the plant cells started to die.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

Histological Study of the Effect of Isoxicam on Ovary of Albino Mice Mus Musculus

Ali Khudheyer Obayes¹, Wurood Mohamed Mutar², Rasha Hamid Ayub³, Sahar Abdullah Mohammed⁴

¹Dep. Biology/ College of Education / University of Samarra/Iraq, ⁴Education of Salahaldin / Dept of Aldor, Tikrit, Iraq

Abstract

Non-Steroidal Anti Inflammatory Drugs (NSAIDs) are the most prescription as therapeutic drugs, used to treat of rheumatic diseases, due to analgesic, antipyretic and anti-inflammatory activity. Isoxicam is a member of NSAIDs group use to stop inflammation, pain associated with arthritis, osteoarthritis, ankylosing and spondylitis. The goal of the present study is to revealed the effect of different doses of Isoxicam on ovaries tissue in mice. Twenty four female mice are randomly divided into control (n = 6) and experimental (n=18) groups. The experimental groups are subdivides into three groups . Each administrated by (0.0714, 0.1428, 0.71428)mg/kg/day for twenty days, respectively; however the control group just injected by distill water. In twenty day, mice were killed and ovaries tissue was prepared for light microscopic examination. All the experimental animals were injected by drug revealed a hyperplasia of germinal cells on the surface of ovary, tongue like projection of primordial oocytes extend to the medulla, multiple oocytes with disarrangement of follicles and deficient of follicular fluid associated with disappearance of oocytes, vacuolation in the cortical layer of the ovary, compressed premature follicle, hypercellularity of follicular cells, degeneration of germinal layer of cortex surface and hyperplasia of primordial oocytes, therefore it is recommended that using of this drug have many side adverse on female fertility.

Key word : Histological , Isoxicam , ovary , albino mice .

Introduction

Isoxicam and Meloxicam these drugs are belonging to the oxicam group. Nonsteroidal anti-inflammatory drugs which display a potent analgesic activity and used for treated rheumatoid arthritis, osteoarthritis and other joint diseases. The pharmacological actions of these oxicam are related to inhibition of cyclo-oxygenase (Cox1,2), an enzyme of prostaglandin biosynthesis at the site of inflammation¹. Prostaglandin, It have been involved as a regulator of several physiological processes in human body such as inflammatory processes in immune response, vasodilator, vasoconstriction, pain perception and fever. Prostaglandin are produced in every tissue of the body (brain, lung, kidney, intestinal digestive system, male and female reproductive system) (²). NSAIDs have many adverse effects of liver, dermis, skin eruption and many physiological disorders in rats’ testis ³-⁶. There are an association between use of prescribed NSAIDs and miscarriage ⁷. The modern NSAIDs that belong to tenoxicam, lonoxicam, Piroxicam and Isoxicam which belongs to oxicam family prescribed as inhibitors of both types of Cox ⁸. A few research consider these drugs a very good antioxidants ⁹. The oxicam family acts on inhibit cyclooxygenases COX-1 and 2, It is also inhibits leucocytes activities.

Fertility in females are affected clearly by COX-2. A study on mice female fertility throughout disruption of COX-2, lead to fails in ovulation, fertilization, implantation, and decasualization. These defects were the direct result of the targeted organ-specific COX-2 deficiency(¹⁰).

The aim of this study was elevate effect of Isoxicam on the ovary after application of different concentration on mice ovary.
Materials and Method

This study was done in medical laboratory department of biology/ Education college/ university of Samarra. Twenty four mature (70 days old) albino Swiss mice Mus musculus Balb/c were employed, weighing (25±3gm) obtained from college of medicine, Tikrit university. They were maintained on 12:12 light: dark bases, and 24 ±2°C with mouse pelleted food and water adlibitum. Female mice were housed in group not bigger than five animals (all from the same experimental group) in plastic cages with metal cover (13*16*30) cm, with wood shavings as bedding material. Twenty four male albino mice were randomly divided into control (n =6) and experimental(n =18) groups. The experimental groups are subdivides into three groups which divided into four groups of mice, each one is injected Intra Peritoneum. with different doses of Isoxicam once daily for 20 days.

Drug administration

Isoxicam ample 200 mg/2ml. Female were injected daily Intra Peritoneum (I.P.) administrated in three doses: Therapeutic dose, over dose1 and over dose2 (0.0714, 0.1428, 0.71428) mg/kg for 20 days respectively 11, and Control group were injected with normal saline 0.9 mg/L.

Surgical procedure

In twenty one days, the female were anesthetized by chloroform, and the peritoneal cavity was opened through a lower transverse abdominal incision. The ovaries was immediately removed and kept in normal saline. At the end the experimental animals were killed by decapitation.

Histological preparation

The collected tissues Each segments of skin was taken and immersed in 10 % formalin foe 24 hours followed by immersion in graded series of alcohol from 70, 80, 90 and 100 %, then clearing with xylene and embedded in paraffin wax at 60 c°. Blocking of the samples were done and the sectioning were performed using a rotary microtome. The thickness of the sections were 6 micrometer. The tissue sections after application of staining with Hematoxylin and Eosin were mounted on the slides using D.P.X and covered by cover slides 12. The slides were examined using light microscope and photographed by manipulated camera prepared for this purpose.

Results

Control group

Histological sections of the ovary in the control group show, intact ovarian surface (germinal layer) directly beneath it are tunica albuginea contain numerous primordial follicles, primary oocytes and secondary oocytes, the cells ranged from flat to cuboidal and low columnar cells. Cortex and medulla regions was continuous, Ovarian follicles are various sizes surrounded by theca interna and theca externa as shown. Each follicle contains a single oocyte in the stroma of the cortex. The oocyte within the secondary oocytes was envelop by the zona pellucida and granulosa cells fig (1).

T1 : Therapeutic group

The histological sections of ovarian cortex in this group shows hyperplasia of germinal cells on the surface of ovary, hyper cellularity in follicular cells around follicular cavity, tongue like projection of primordial oocytes extend to the medulla fig (2A), multiple oocytes with disarrangement of follicles and deficient of follicular fluid associated with disappearance of oocytes , hyperplasia of follicular cells primordial oocytes fig (2B).

T2 : Over dose 1

This group showed degeneration of germinal cells layer on the surface of cortex with thickening of collagen fibers around premature oocytes, disappearance of oocyte with vacuolation in the cortical layer of the ovary fig (3A). In other sections showed hypercellularity of follicular cells of oocytes in the cortex , degeneration of follicular cells and secondary oocytes with vacuolation of interstitial connective tissue fig (3B). In tertiary follicle increased a multiple layer of follicular cells around oocytes, disappearance of oocytes with decreased amount of follicular fluid and detachment of follicular cells from surrounded connective tissue fig (3C) .other sections of cortex demonstrated compressed premature follicle , degeneration of germinal layer of cortex surface and hyperplasia of primordial oocytes fig (3D).

T3 : Over dose 2

In this group the microscopical examination were show degeneration of germinal epithelium cells of cortex surface, vacuolation in granulosa layers of premature follicle with remnant of oocyte of premature follicle fig (4A). In the surface of cortex showed
disappearance of germinal epithelium, disarrangement of the other primary follicles with compressed it and partial congestion of blood vessels fig (4B). In other section showed degeneration of follicular cells layer in premature follicle hyper cellularity of connective tissue and vacuolation in between collagen fibers fig (4C). In medulla of ovary showed empty of blood vessels, spaces in between follicular connective tissue, and hypertrophy of the stromal cells fig (4D).

Fig (1) primordial follicles (1), primary oocytes (2), secondary oocytes (3) and cortex (4). H&E, 40X.

Fig (2): hyperplasia of germinal cells (1), hyper cellularity in follicular cells (2) and tongue like projection of primordial oocytes (3). A. multiple oocytes with disarrangement of follicles with disappearance of oocytes (1), hyperplasia of follicular cells (2) B. H&E, 40X.

Fig (3) degeneration of germinal cells layer (1), vacuolation in the cortical layer (2), thickening of collagen fibers around premature oocytes (3) disappearance of oocyte (4). A. hypercellularity of follicular cells (1), degeneration of follicular cells (2), vacuolation of interstitial connective tissue (3) B. increased a multiple layer of follicular cells (1), disappearance of oocytes (2), decreased amount of follicular fluid (3) and detachment of follicular cells from surrounded connective tissue (4) C. compressed premature follicle (1), degeneration of germinal layer of cortex surface (2) and hyperplasia of primordial oocytes (3). D.
Discussion

The present study was designed to demonstrate the effect of Isoxicam after application on mice for 21 continuous days, so the Isoxicam was intraperitoneal injection for three different doses. The application of this drug demonstrated many histopathological changes in ovaries was affected at different degrees, which means that increasing the concentration lead to severely effect. The female reproductive system is considered to be the most important organ. It characterized by two main functions, synthesis of sex hormones and produce the oocytes (13). Non-steroidal anti-inflammatory drugs (NSAIDs) are the more effectively to reduce pain and inflammation (14).

COX-2 produce prostaglandin which plays a major role in ovulation and fertility. NSAIDS or COX-2 inhibitors which effect on follicle rupture, ovulation, fertilization, luteolysis and parturition when treated rats with indomethacin (15).

Our data was in agreement with (16) who described the effect of ibuprofen at therapeutic dose in mice which induced a histological alteration such as a sequence of events of development and growth of ovarian follicles, increased the number of atretic follicles, Degenerated oocytes of matured follicles with vacuolated stroma. The administration Sodium Metabisulfite on rats which induced histological changes represented by reduced volume of the ovary as well as a decrease in the number of growing follicles, corpus luteum and an increase in the number of atretic follicles. Due to increased lipid peroxidation in the ovarian tissue (17) this study was agreement with our results. The administration of tarragon extract flavonoids reduces cyclooxygenase enzyme and nitric oxide, and thus reduces the amount of prostaglandin which effect on follicle growth (18). Oral administration with indomethacin causes ovulatory dysfunction, represented by the occurred of abnormal follicles at the, with degenerated granulosa cells and reduced follicular fluid of secondary follicle, all these
defect due to COX-2/ prostaglandin synthesis inhibition.

Treated rats by atropine sulphate was induced degeneration of granulosa cells and disappearance of antrum the inhibition gonadotrophin release and prostaglandin synthesis. The application of this drug was indicated the insult of Isoxicam in any concentration even therapeutic dose, so our suggestion that this drug must not be used by owner, just used by supervision of the doctors.

Conclusion

The present study revealed that Isoxicam caused a clear histological alterations in ovarian tissue, including hyperplasia of germinal cells on the surface of ovary, hyper cellularity in follicular cells around follicular cavity, tongue like projection of primordial oocytes extend to the medulla, disappearance of oocytes with decreased amount of follicular fluid, vacuolation in granulosa layers of premature follicle with remnant of oocyte of premature follicle. Therefore it is recommended that usage of this drug have harmful side effects on female fertility.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References


**Genotyping of Tumor Necrosis Factor-α in Inflammatory Bowel Iraqi Patients**

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**Abstract**

The TNF-α gene considered as strong candidate for immune modulator and pro inflammatory cytokine responsible for genetic susceptibility of chronic disease such as the initiation and development of inflammatory bowel disease (IBD). The aim of this study is to investigate the genetic polymorphisms of -1031 in TNF-α gene with susceptibility of Iraqi IBD patients.

**Method:** The total number of this study 95 blood samples (75 Iraqi IBD patients and 20 from healthy individuals as a control group). The genetic polymorphisms in TNF-α-1031 gene was investigated using restriction fragment length polymorphism (RFLP) and Sanger sequences techniques.

**Results:** The genotype allele frequency of -1031 polymorphism was significantly higher in CD Iraqi patients (\(P=0.042\), Chi 4.612. OR= 0.72-1.64). C allele may be have protective role, whereas the T allele may increase susceptibility to IBD.

**In conclusion:** The TNF-α -1031 gene polymorphisms in promoter region have an important role in the occurrence of inflammatory bowel disease of Iraqi population especially in CD patients although some results didn’t give us a significant differences. It’s possible that C allele may be have protective role, whereas the T allele may increase susceptibility to IBD.

**Key words:** TNF-α -1031, Tumor necrosis factor, inflammatory bowel disease, ulcerative colitis and Crohn’s disease.

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**Introduction**

IBD include both Crohn’s disease (CD) and ulcerative colitis (UC). Patients with IBD suffer from some common symptoms such as acute diarrhea, abdominal pain, fatigue and weight loss. The location of the two type of inflammation is different, it affects entire gastrointestinal tract in the CD where the UC affects the ileum and colon. Environmental factors, genetic and immune regulation play a key role in development and progression of IBD which characterized by an irregular immune response of the mucous layer in the intestine to bacterial antigens within the intestinal lumen. Regulation of cytokines such as TNF-α and IL-6 play a key role in activation of T helper cells which causes inflammation disease. TNF-α is the important cytokine for inflammation as it participates in immune response to IBD. TNF-α characterized with a wide range of inflammatory activity it is usually produce by macrophage and monocyte although there are other types of cells that are produced but in limited quantities. Inflamed mucous layer of the intestine in IBD patients have increased gene expression of TNF-α gene. TNF-α not only stimulates the acute stage of inflammation but plays role for the occurrence apoptosis, proliferation and differentiation of cells and several immune disorders.

There is a correlation between the genetic polymorphisms of the encoded gene of TNF-α and the susceptibility to IBD. The TNF-α - gene have shown several polymorphisms in its four exons. However, most of the common reported polymorphisms are identified in the promoter region of this gene. The TNF-α promoter region include various a single nucleotides...
polymorphisms that have shown a significant association with IBD\(^{(11)}\), like \(\text{TNF-}\alpha\ -(308G/A)\)\(^{(12)}\) and \(\text{TNF-}\alpha\ -857\) polymorphism\(^{(13)}\). The response to anti TNF-\(\alpha\) agents is increased and reduced by the effect of genetic factors of individuals\(^{(14)}\).

The aim of this study is to detect the \(\text{TNF-}\alpha\ -1031\) gene polymorphisms as its important factor in IBD development and treatment.

**Materials and Method**

**Patients:**

Blood samples were obtained from 75 Iraqi patients suffering of IBD who were attending the Gastroenterology and Hepatology Disease Center in Baghdad between September–Desember, 2018. Patients samples were selected after the diagnosis was made by the specialist doctor, in addition to 20 blood samples were collected from apparently healthy individuals. Ethical permission to conduct the research was obtained from this hospital and from all participants in this study.

**DNA Extraction**

Genomic DNA was isolated from blood sample according to the instructions of ReliaPrep™ Blood gDNA Miniprep System kit (Promega, USA).

**Polymerase chain reaction (PCR)**

PCR was done for \(\text{TNF-}\alpha\ -1031\) gene amplification, each 20 \(\mu l\) mixture of PCR reaction include 10 \(\mu l\) of master mix, 1 for each primer and 5 distilled water and 3 of genomic DNA. The forward and the reverse primers were 5’-TATGTGATGGACTCACCAGGT-3’ and 5’-CCTCTACATGGCCTGTCTT-3’ respectively\(^{(15)}\).

PCR amplifications were achieved in Thermal cycler (Applied Biosystem 96). PCR reactions were started through initial denaturation at 95 °C for 5 min followed by 30 cycles of denaturation at 95 °C for 30 s., annealing at 55 °C for 30 s. and extension at 72 °C for 30 s. followed by a final extension at 72 °C for 7 min. then hold at 4 °C.

**Restriction Fragment Length Polymorphism (RFLP):**

PCR products were digested with restriction enzyme, 1\(\mu l\) from BbsI enzyme (Biolabs, England) was added to 5\(\mu l\) of PCR product for each sample. RFLP was performed using Thermal Cycler (Bio Rad, USA) with the following temperature program: 37 °C for 3 hour, enzyme inactivation at 65°C for 5 minutes followed by 10 min incubation at 4°C to stop the reactions. The restriction enzymes fragments were separated on 2% agarose gel stained with ethidium bromide stain.

**Sequencing**

PCR products were send to Macrogen Corporation – Korea for Sanger sequencing using (ABI3730XL, automated DNA Sequencer). The results were analyzed using genious software.

**Statistical analysis**

The Hardy-Weinberg equilibrium was used by the chi-square test to evaluate the frequency of genotypes and correlation of the \(\text{TNF-}\alpha\ -1031\) genotypes or alleles between IBD patients and controls group. Calculated Odds ratio (OR) with 95% confidence interval (95% CI) for assessing the correlation strength. All data were analyzed using SPSS (2012). A \(p\) value of <0.05 was considered significant\(^{(16)}\).

**Results and Discussion:**

**Clinical Characteristic of Patients**

The total number of this study was 95 blood samples including 75 blood samples from IBD Iraqi patients in addition to 20 blood samples from healthy individuals as a control group. The IBD patients whose enrolled in this study divided into two groups: the first group 47 UC patients and 28 CD patients. Table 1 refer to the clinical characteristics of IBD patients, the ages where ranged between (19-57) years. High rate of patients less than 50 years (81%) with IBD were found in this study while the older age group or more than 50 years was (19%) with high significant association (\(P<0.01\)). This result was agreed with\(^{(17)}\) they stated that the peak occurrence of the IBD occurs in the second or third decade of life. High incidence of IBD in females, it was (57%) compared with (43%) in males, significant association (\(P<0.05\)), was appear depend on patients gender. Statistically significant differences were observed between IBD patients and controls depend on type of disease, patients with UC were (60%) higher than patients with CD (40%), these results consist with other Iraqi study from Erbil city\(^{(18)}\), this data may be indicate to that UC has more prevalent than CD in Iraqi population.
Table 1: Clinical Characteristics of IBD Patients

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Total No. and Percentage %</th>
<th>Chi-Square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 ≥</td>
<td>14(19%)</td>
<td>13.208 **</td>
</tr>
<tr>
<td>50 &lt;</td>
<td>61(81%)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>32(43%)</td>
<td>5.017 *</td>
</tr>
<tr>
<td>Female</td>
<td>43(57%)</td>
<td></td>
</tr>
<tr>
<td>Type of disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UC</td>
<td>45(60%)</td>
<td>7.250 **</td>
</tr>
<tr>
<td>CD</td>
<td>30(40%)</td>
<td></td>
</tr>
</tbody>
</table>

Genotyping of \textit{TNF-}\alpha - 1031 gene and Alleles frequency in IBD patients

The genetic polymorphisms in \textit{TNF-}\alpha gene considered as strong candidate for immune modulator and pro inflammatory cytokine responsible for genetic susceptibility of chronic disease and the initiation and expansion of IBD \cite{19}.

The genetic polymorphisms in the -1031 (T→C) of \textit{TNF-}\alpha gene promoter (db SNP accession number 1rs1799964) was investigated in Iraqi patients with IBD by PCR-RFLP technique and Sanger sequences to determine the genotypes/allele frequency, at this site there are three genotype of \textit{TNF-}\alpha T-1031C in promoter was found , TT with band sizes 251bp and other small band 13 pb that emerge with primer dimer while TC and CC that have band sizes (251/180/ 71) bp and (180/71) pb respectively Figure 1. The sequences analysis of \textit{TNF-}\alpha – 1031T>C showed in Figure 2.

Figure 1: Genetic polymorphism in T -1031 C of TNF-\alpha gene on 2% agarose gel with ethidium bromide dye after digestion with Bbsl enzyme, M : DNA molecular size marker (100bp) , Lane (11) TT genotype (251/13) bp , Lanes (16,20) TC genotype (251/180/ 71) bp and line (25) CC genotype (180,71)bp.
The frequency distribution of genotypes and alleles of \(TNF-\alpha\)-1031 in IBD patients and control groups are summarized in Table 2. The present results showed 62.7\% vs 70\% \((p=0.061, \text{Chi}=3.091 \text{NS, OR}=0.377)\) for homozygous T allele in IBD patients and control group, the homozygous CC genotype was (5.33\% vs 5\%) in IBD patients and control group \((p=0.872, \text{Chi}=0.055 \text{NS, OR}=0.0162)\) The T>C heterozygous genotype in IBD patients (32\%) was higher ratio than control(25\%) \((p=0.069, \text{Chi}=2.983, \text{OR}=0.352)\) the data showed no significant difference was found in correlation to the three genotypes, our results agreed with Iranian population \(^{(20)}\).

Alleles frequency for T and C alleles in IBD patients and control group were (0.97, 0.83) and (0.21, 0.17) respectively. The results revealed that there was no significant association regarding genetic polymorphism in \(TNF-\alpha\) (-1031T/C) polymorphism in IBD patients, statistical analysis indicated that \(TNF-\alpha\) (-1031T/C) was not a risk factor to IBD.

**Table 2: Genotyping of \(TNF-\alpha\) - 1031 gene in IBD patients**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patients IBD N (%) 75(100%)</th>
<th>Control N (%) 20 (100%)</th>
<th>(P)-Value</th>
<th>Chi-Square</th>
<th>OR (CI)</th>
<th>EF</th>
<th>PF</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>47 (62.67%)</td>
<td>14 (70.00)</td>
<td>0.061</td>
<td>3.091 NS</td>
<td>0.377 (0.78-1.49)</td>
<td>0.511 ***</td>
<td></td>
</tr>
<tr>
<td>TC</td>
<td>24 (32.00%)</td>
<td>5 (25.00)</td>
<td>0.069</td>
<td>2.983 NS</td>
<td>0.352 (0.82-1.55)</td>
<td>*** 0.376</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>4 (5.33%)</td>
<td>1 (5.00)</td>
<td>0.872</td>
<td>0.055 NS</td>
<td>0.0162 (0.69-1.58)</td>
<td>*** 0.152</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Allele Frequency</th>
<th>T</th>
<th>0.79</th>
<th>0.83</th>
<th>--</th>
<th>--</th>
<th>--</th>
<th>--</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>0.21</td>
<td>0.17</td>
<td>--</td>
<td>--</td>
<td>--</td>
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</tr>
</tbody>
</table>

**NS:** Non-Significant.

OR = Odd Ratio CI = Confidence Interval EF = Etiology fraction PF= Preventive fraction
Our study demonstrate that the TT genotype has clearly indicates an etiology for IBD, as it had an OR of 0.377 and Etiologic Fraction (EF) of 0.511 (Table 3), in contrast, the TC and CC genotype have rather preventive role as it had Protective Fraction (PF) of .376 and 0.152 respectively with low OR (0.352 and 0.0162) .With the possibility of C allele may be have protective role , whereas the T allele may increase susceptibility to IBD.

Genetic polymorphisms of TNF-α gene in UC patients

Genotype of the TNF-α genes (-1031) polymorphisms in 45 UC patients (Table 3) .The data revealed that homozygous TT genotype was found 63.83% vs 70% in UC patients and control group respectively (p=0.067,Chi=3.055 NS,OR=0.362) while homozygous CC genotype in UC patients and control group 6.38% and 5% respectively .Heterozygous T>C genotype in UC patients(29%) was higher than control group (25%) however , the three genotypes in current work didn’t give significant differences . The heterozygous T>C genotype in this study similar with (21).

Our results also similar to data by Asghar and his colleagues (22),they investigated the possible association between five single nucleotide polymorphism (SNPs) in TNF-α gene promoter polymorphisms including -1031T/C in a Japanese population with endometriosis, their results revealed that –1031 (65.1%) TT , (31.7%) TC, (2.8%) CC, (81.4%)T (18.6%)C, the -1031C polymorphism with no significant difference in the frequency of in the TNF-α-1031 gene promoter .

The T allele in promoter region of TNF-α gene at -1031 site gave a significant risk for development in Turkish and Iranian IBD especially UC patients and also found that C allele was very low in patients and could have a protective role ,the variation in TNFα-1031 T allele may increase the risk for developing UC , single nucleotide polymorphism -1031 T > C could have important effect in pathogencity of IBD that lead to increase TNF-α levels in IBD patients (23). Increased expression of TNF-α , high serum levels have been documented in intestinal tissues and IBD patients (24).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>UC Patients N (%)</th>
<th>Control N (%)</th>
<th>P-Value</th>
<th>Chi-Square</th>
<th>OR (CI)</th>
<th>EF</th>
<th>PF</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>30 (63.83)</td>
<td>14 (70.00)</td>
<td>0.067</td>
<td>3.055 NS</td>
<td>0.362</td>
<td>(0.84-1.58)</td>
<td>0.269 ***</td>
</tr>
<tr>
<td>TC</td>
<td>14 (29.79)</td>
<td>5 (25.00)</td>
<td>0.080</td>
<td>2.741 NS</td>
<td>0.279</td>
<td>(0.78-1.62)</td>
<td>*** 0.084</td>
</tr>
<tr>
<td>CC</td>
<td>3 (6.38)</td>
<td>1 (5.00)</td>
<td>0.706</td>
<td>0.051 NS</td>
<td>0.0158</td>
<td>(0.83-1.62)</td>
<td>*** 0.081</td>
</tr>
</tbody>
</table>

Allele Frequency

<table>
<thead>
<tr>
<th>Allele</th>
<th>T</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>0.79</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>0.83</td>
<td>0.17</td>
</tr>
</tbody>
</table>

NS: Non-Significant.

Table 3: Genotyping of TNF-α-1031 gene in UC patients

Conclusion

This paper suggests that TNF-α -1031 gene polymorphisms in promoter region has an important role in the occurrence of inflammatory bowel disease of IBD patients in Iraqi population especially in CD patients although some results didn’t give us a significant differences. It’s possible that C allele may be having protective role, whereas the T allele may increase susceptibility to IBD. Other studies are needed with
large number of IBD patients to support our results.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** Self-funding

**References**


Antibacterial activity of Eruca Sativa Seeds Aqueous Extract Against Human Pathogenic Bacteria

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Abstract

This research was achieved with the aim of detecting the Antibacterial efficacy of the watery extract Eruca of Sativa seeds toward human pathogenic bacteria, four Pathogenic species of bacteria (Enterococcus faecalis, staphylococcus aureus, pseudomonas aerogenoso, and Salmonella typhi) were isolated from different clinical samples at al. Kademia teaching hospital Laboratory. Results: The results showed The extract inhibits gram positive bacteria and the mean inhibitory zone for strepto coccus faecalis and S. aureus was 10.4 mm and 14.0 mm respectively. Conclusion: The extract had no effect on gram negative bacteria.

Keywords: Eruca Sativa, seeds aqueous extract, pathogenic Bacteria

Introduction

Eruca sativa Miller (synonym Eruca vesicaria Rocket) , usually called as Tarmira, Garden salad or Rocket salad or Jarjeer. E. sativa is one of the endemic species from the family of Brassicaceae which is cultivated in most cases in countries of Mediterranean sea like Greece, Turkey, and Italy. It is an annual dark-green plant, with height around 20-50 cm, with a taste of spicy pungent (1). Its seed is commonly yellow, but sometimes is reddish yellow or spotted with brown-green spots (2).

The seeds have long been used in folk medicine as a lactagogue, aphrodisiac, diuretic, antis -corbutic, antimicrobial, to disintegrate renal calculi and induce vomiting

This used seeds were taken long time in traditional medicine as an aphrodisiac, diuretic, lacagog, antimicrobial, anti-bacterial, to induce vomiting and destroy kidney stones (3). However, Flanders and Abdel-Karim (4) indicated that oil of seeds of Eruca sativa contains 93.8% fatty acids (11 ones) including 58.5% erucic acids, 6.7% saturated acids, 28.5 % linoleic acid, 1-2 % linolenic acid and 4.5% oleic acid. Depending to to EL-Gendy (5), oil of Eruca sativa rises count of RBCs and the content of haemoglobin.

S. aureus is one of the Gram-positive bacteria, has a coccus shape and it is usually considered as a member of the microorganisms in the human body frequently. It is a member of the Firmicutes and it is exist on the human skin and in upper tracts of the respiratory system (6).

Usually, Staphylococcus aureus behaves the commensalism role, atypically colonizing approx. 30% of the population of humans. It can occasionally cause some diseases. Particularly, it is mostly one of the common pathogens of infective endocarditis and bacteremia. In addition to, it can lead to different infections of soft tissues and skin, especially when mucosal barriers or the skin have been penetrated (7).

Pseudomonas aeruginosa have a common capsule, bacili-shaped, Gram-negative bacterium which can leads to occurrence some diseases in humans, animals, and plants. A species of great medical significance.
Pseudomonas aeruginosa is an opportunistic pathogen in hospitals for individuals with immunodeficiency. It archetypically infects burns, urinary tract, the airway, wounds, and other infections of the blood.

This is the most frequent cause of the infection of external ear (otitis externa), injuries, and burns, which is the most common colonizer of the medical devices such as catheters. P. aeruginosa can be spreading by the contaminated equipments and isn’t carefully cleaned or by the hands of health care workers. It can rarely cause pneumonia acquired from the community (8).

Salmonella typhi, the Gram-negative bacterium also called as S. enterica serotype Typhi, which grow in blood and intestines. Typhoid fever or Typhoid is spreading by drinking water or consuming food contaminated with stool of the infected people.

Typhoid is an infectious bacterial disease caused by S. typhi which causes differential symptoms. Symptoms may vary from severe to moderate and always start 6-30 d after the exposure to this bacteria (9).

**Objective**

This study was carried out with the aim of detecting the antibacterial efficacy of the watery extract of Eruca Sativa seeds toward some pathogenic bacteria to humans.

**Material and Method**

a. **Bacterial isolates:**

Four Pathogenic species of bacteria were isolated from different clinical samples at al. Kademia teaching hospital Laboratory.

The isolates were Gram positive coca, Enterococcus faecalis and staphylococcus aureus (S. aureus). And Gram negative loacilli, pseudomonas aerogenoso, and Salmonella typhi.

Bacteria were isolated and diagnosed according to cultural, morphological and biochemical characters according to (10).

b. **subculturing of bacterial isolates**

From stock culture, Nutrient broth tubes were inoculated for each bacterial species separately, and the tubes incubated at 37 °C for 24 h.

d. **susceptibility test:**

The susceptibility of the seed aqueous extract was determined by disc diffusion method according to (11).

From stock culture, Nutrient broth tubes were inoculated for each bacterial species containing approximately 10^7 cfu / mL was transferred aseptically and spread on the surface of plates of MullerHinton agar (MH-agar).

Serile discs prepared from the filter paper (6 mm) were impregnated with 20 micro liters of the extract, a standard antibiotic disc gentamicin 40 mg/ml has been used as a positive-control and a disc containing 20 micro liters of sterile distilled water as a negative-control. However, all the done discs have been placed on the surface of MH medium and then the dishes have been incubated at 37 °C for 24 hr. The test was done in triplicate for each bacterial species.

**Results**

The average of inhibition Zone diameter was determined after the end of the incubation period.

The extract inhibits gram positive bacteria and the mean inhibition Zone for strepto coccus faecalis and S. aureus was 10.4 mm and 14.0 mm respectively.

The extract had no effect on gram negative bacteria, the results were shown in table 1.

**Table (1) Antibacterial efficacy (zone of inhibition) of seeds extract of E. sativa (mm)**

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Aure of isolate</th>
<th>E. sativa extract</th>
<th>Gentamicin positive control</th>
<th>Distilled water negative control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus faecalis</td>
<td>Urine</td>
<td>10.4</td>
<td>25.3</td>
<td>-</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Wound</td>
<td>14.0</td>
<td>22.0</td>
<td>-</td>
</tr>
<tr>
<td>Pseudomonas aerogenosa</td>
<td>Burn</td>
<td>-</td>
<td>15.8</td>
<td>-</td>
</tr>
<tr>
<td>Salmouella typhi</td>
<td>Stool</td>
<td>-</td>
<td>18.0</td>
<td>-</td>
</tr>
</tbody>
</table>

- : means no inhibition zone.
Discussion

The increasing occurrence of resistant bacteria could be due to overuse or misuse of commercially available antimicrobials in addition to side effect and toxicity of some of these antimicrobials, this made scientist try to develop aneefective, alternative, affordable, safer and nontoxic antimicrobials of plant origin.\textsuperscript{(11, 12)}.

Eruca sativa seed extract contains several secondary mrtabolites [ glucosinolate isothiocyanate, Alkaloids, and theic derivaties, Flavonoid, phenols, Tannins, and erucin which are responsible for their antioxidant and antibacterial activity.\textsuperscript{(13, 14)}.

Enterococcus Faecalis and S. Aurus was sensitive to the extract and this may be due to mesh like peptidogly can Layer in their cell wall\textsuperscript{(15)}. While Pseudomonas aerogenosa and Salmonella typhi were resistaut to the extract due to the selective permeability of Lipopoly saccharide membrane to hydrophilic solutes which restrict the entry of the extract\textsuperscript{(16)} and the extraction method used and type of solvent may affect the result too.\textsuperscript{(17, 18)}.

The absence of antimicrobial activity of the extract does not indicale that the plant was inactive or not contain bioactive substances\textsuperscript{(19)}.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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Possible Role of Toxoplasmosis on Gene Sequence Alteration in Patients with Cardiovascular Diseases

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Abstract

Toxoplasmosis is one of the risky infection my lead to cardiovascular diseases. One hundred therein patients samples were collected with Heart Diseases and there were infected with Toxoplasmosis, in Baghdad educational Hospital from period 1st January 2019 to 1st September 2019. The results show that prevalence of *Toxoplasma gondii* among Hypertensive disease and Myocardiopathy with Heart Diseases. The genotype of *MYLK3* exon to Human cardiac gene by gel electrophoresis, Lane M markers correspond to 500 bp ladder lane 2 of gene band with 600bp. Mutation occurrence in exon 8 the gene sequence CCCAGCCGG were change to CCCATCCGG, and in exon 9 the change occurrence in sequence CTCAAGCGG to CTCAAGGTACAA.

**Keywords:** *Toxoplasma gondii, Alteration, gene sequence, cardiovascular diseases.*

Introduction

Toxoplasmosis is generally a mild infection with signs of lymphadenopathy, but some patients may develop chorioretinitis which can progress into blindness(1). Severe neurological disorders may be shown by those immunocompromised patients who are infected with *Toxoplasma gondii* (2). Congenital disorders of newborns may also result from primary toxoplasmosis during pregnancy (3). In humans, heart may be affected by toxoplasmosis (4) with myocarditis (2), myocarditis with pericarditis (21, 22) as well as the acute heart failures (5). Patients with toxoplasmosis who develop myocarditis may present with congestive heart failure, arrhythmias, pericardial effusion and constrictive pericarditis (2). There are few studies on the sero-epidemiology of patients suffering from heart diseases due to toxoplasmosis (6). Our study aimed to determine the association between exposure to *Toxoplasma gondii* and patients with cardiac diseases who attended to Baghdad teaching hospital/ Medical city and the association between seropositive patients and behavioral, demographic as well as the clinical features of patients.

Materials and Method

One hundred therein patients samples were collected with Heart Diseases and there were infected with Toxoplasmosis, in Baghdad educational Hospital from period 1st January 2019 to 1st September 2019, all these patients were diagnosed with Toxoplasmosis by ELISA, Anti *Toxoplasma* antibodies IgM and IgG. Genetic test were done by conventional PCR Primers used for amplifying *MYLK3* exons

AGCTGGGCCCTCTCTTT
CCTGGCATCAGACTGCACC
GTGCCGGGAGACCTGGGTTTGA
CCTGCCGGTGACTCTGCTCTAA

Statistical analyzing

Preceded data has been entered to the computer with the use of “Statistical Package of Social Science” Software program, v. 18 (SPSS).
Results

The association between toxoplasmosis seroprevalence and the clinical features of patients with cardiac diseases:

Table (1): The prevalence of *Toxoplasma gondii* among Hypertensive disease and Myocardiopathy with Heart Diseases.

<table>
<thead>
<tr>
<th>Features</th>
<th>Patients’ Number</th>
<th>Prevalence of toxoplasmosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>yes</td>
<td>25</td>
<td>3</td>
</tr>
<tr>
<td>No</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>No</td>
<td>24</td>
<td>9</td>
</tr>
<tr>
<td>Myocardiopathy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>No</td>
<td>38</td>
<td>5</td>
</tr>
</tbody>
</table>

Mutation occurrence in exon 8 the gene sequence CCCAGCCGG were change to CCCATCCGG, and in exon 9 the change occurrence in sequence CTCAAGCCGGAG to CTCAAGGTACAA of *MYLK3* gene shows in table 2.

Table (2): 2 Mutation occurrence in exon 8 and exon 9.

<table>
<thead>
<tr>
<th>Reference CCCAGCCGG</th>
<th>Variant (c.1915-1G&gt;T) in Exon 8</th>
<th>CCCATCCGG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference CTCAAGCCGGAG</td>
<td>Variant (Exon 9)</td>
<td>CTCAAGGTACAA</td>
</tr>
</tbody>
</table>

Discussion

Toxoplasmosis is one of the risky infection my lead to cardiovascular diseases. Cardiovascular diseases are common conditions in adults that may be in the heart muscles or vessels. *Toxoplasma gondii* due to its presence within the cellular tissue may affect the heart. 12% of people with Hypertensive disease are infected with Toxoplasmosis and they are mainly cardiovascular disease. These finding matched with (Flegr, J. *et al*, 2014) who found that 24% of cardiovascular diseases complaining hypertension and they have toxoplasmosis. 22.2% of myocardiopathy patients suffer from cardiovascular patients, also they have toxoplasmosis. This report agreed with (England, J. H. *et al*, 2019), who reported that 26% with cardiovascular disease and they complaining myocardiopathy with Toxoplasmosis. Cardiovascular disorders are heart conditions, this
also result from the weakened heart (9). The tropical known as arrhythmias. Heart valve disorders may and it may lead to heart failure or irregular heartbeats normal electrical rhythm also becomes less than usual, the heart becomes less throughout the body and maintaining the heart becomes weaker. Pumping of blood by the conditions, the ill tissue of the cardiac muscle is replaced with scar tissues. When cardiomyopathy worsens, then the heart becomes weaker. Pumping of blood by the heart becomes less throughout the body and maintaining normal electrical rhythm also becomes less than usual, and it may lead to heart failure or irregular heartbeats known as arrhythmias. Heart valve disorders may also result from the weakened heart (9). The tropical pulmonary eosinophilias, which have a characteristic of restrictive lung disease and progressive interstitial fibrosis, can lead to PH and then to a course of filarial infection. Intracardiac rupture of Echinococcus cyst and Toxoplasma gondii may lead to the membrane or secondary cyst embolization of the organs or lungs that are supplied by the systemic circulation. cardiac involvement by parasites must be considered in the differential diagnosis, despite unusual reasons of heart diseases outside the endemic areas, especially in myocardial or pericardial patients of unknown causes. In this study, the present knowledge on the main cardiac diseases caused by the protozoan and metazoan parasites have been updated and summerized, including the heart muscle either directly adversely, (Nunes, M. C. P et al, 2007), (10). The genetic mutation of cardiovascular disease patients associated with toxoplasmosis has been found to exacerbate the pathological condition and alter the gene trajectory of the infected. These findings were in harmony with (Webster, J. P. et al, 2013) who reported the behavioral alterations seen in the infected hosts indicate the following: (1) The active manipulation of the parasite’s selective benefit; (2) the active manipulation of the host’s selective benefit to improve the effects of the infection; (3) The general pathological response of the host of no clear parasite’s or host’s selective benefit; or, finally, the subtle distinction of the latter grouping known here as (4) the ‘by-product pathology’ as a result of the accidental toxoplasmosis selected for behavior manipulation in the alternative host species or the stage of the life cycle. In addition, as this perspective study evaluates the applicability of studying Toxoplasma gondii in rats (and/or mice), the intermediate hosts as models to help us understand both evolutions and mechanisms that underpin parasite-changed behaviors (ranging from rodent’s predation to some conditions of human schizophrenia), for the first time in this review we have introduced the novel term of ‘T. gondii–rat manipulation–schizophrenia model’ (11). The genetic mutations that occurred in exon 8 and exon 9 on the MYLK3 gene proved that there was a genetic sequence change in this muscle tissue of the heart due to the involvement of Toxoplasma gondii in the exacerbation of the disease. These work agreed with (Ngô, H. M. et al.2017) who reported that these data were de convoluted using three biology system topics: “Orbital deconvolution” elucidated upstream, regulatory pathway interconnecting human susceptible genes, biomarkers, proteomes in addition to transcriptomes. “Cluster deconvolution” showed visual protein-protein interactions included in a process that affects brain function and circuitry, such as leukocyte migration, lipid metabolism and olfaction. Eventually, “disease deconvolution” which identifies correlations between epilepsy and parasite-brain interactions, movement disorders, Alzheimer’s as well as cancer. This “reconstruction-deconvolution” provides templates of the progenitor cells’ potentiating influences and components affecting human brain parasitism and disorders (12). Little research has been done on the incidence of people suffering from heart disease may be involved in Toxoplasma or may be conducive to these injuries and therefore has been proven that there is a direct relationship with Toxoplasma infection and cases of heart disease and this report works for the first time in Iraq.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** Self-funding
References


Study the Cytochrome P450 Gene Expression Changes in Iraqi Patients with Chronic Liver Disease

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Abstract

The cytochrome P450 is a chemical group of heme-containing proteins which speaks to one of the biggest and most practically various superfamilies’ found in nature. Chronic liver disease (CLD) is a process of the liver that involves a process of progressive destruction and regeneration of the liver parenchyma leading to fibrosis and cirrhosis. The aim of this study was to assess the cytochrome P450 gene expression changes of three variants, CYP1A2, CYP2B6, and CYP2E1 in Iraqi patients with chronic liver disease (hepatitis+ alcoholism and hepatitis+ non-alcoholism). CYP1A2, CYP2B6, and CYP2E1 mRNA gene expression were assessed by quantitative real-time PCR (qRT-PCR) in 50 cases with CLD and 50 subjects as controls. Primers for genes of interest, CYP1A2, CYP2B6, and CYP2E1 mRNA and housekeeping gene (GAPDH) were designed using NCBI tools. Fatty acid synthase (FAS) was estimated by ELISA technique. The results showed statically significant differences in FAS levels (ng/ml) between study groups, HC, HNC, and control (p-value < 0.05). GEF in CYP1A2 gene showed no change between HC and control but with statistical variations in GEF in HNC and both HC or control. GEF in CYP2B6 and CYP2E1 genes showed highly significant differences between control and both HNC and HC. In conclusion, CYP2B6 and CYP2E1 gene expression were risk factor for progression HC but not HNC through stimulation of increasing levels of FAS.

Keywords: chronic liver disease, cytochrome P450, Fatty acid synthase, gene expression.

Introduction

The cytochrome (P450) is a chemical group of heme-containing proteins which speaks to one of the biggest and most practically various superfamilies’ found in nature (1). The primary capacity of P450s is to encourage the biotransformation of mixes by expansion of practical gatherings reasonable for conjugation and extreme disposal from the life form. The aim of this study was to assess the cytochrome P450 gene expression changes in patients with chronic liver disease (2). In the clinical context, Chronic liver disease (CLD) is a process of the liver that involves a process of progressive destruction and regeneration of the liver parenchyma leading to fibrosis and cirrhosis (3). Patients with either diagnosed or undiagnosed chronic liver disease occasionally present with an acute deterioration of liver function caused by direct or indirect insults to the liver (4). The expression of CYP450 enzymes is influenced by endogenous factors such as genetic polymorphisms, gender, age, and the levels of endocrine hormones (5). The expression of CYP45 enzymes is also influenced by exogenous factors such as drugs and environmental chemicals, as well as the physico-pathological conditions (6). CYP1A2 is a member of the cytochrome P450 with a mixed-function of oxidase system, it is involved in the metabolism of xenobiotics in the body and in humans, the CYP1A2 enzyme is encoded by the CYP1A2 gene (7). This quality, CYP2B6, encodes an individual from the cytochrome P450 superfamily of proteins. The cytochrome P450 proteins are monooxygenates which catalyze numerous responses engaged with medication digestion and amalgamation.
of cholesterol, steroids and different lipids. This protein restricts to the endoplasmic reticulum and its appearance is initiated by phenobarbital. The catalyst is known to use some xenobiotic, for example, the counter malignant growth drugs cyclophosphamide and ifosphamide (8). CYP2E1 is a layer protein communicated in significant levels in the liver, where it makes about half out of the absolute hepatic cytochrome P450 mRNA(7) and 7% of the hepatic cytochrome P450 protein.[8] The liver is thusly where most medications experience deactivation by CYP2E1, either legitimately or by encouraged discharge from the body. CYP2E1 uses for the most part little, polar particles, including dangerous research facility synthetic substances, for example, dimethyl formamid, aniline, and halogenated hydrocarbons. While these oxidations are frequently of advantage to the body, certain cancer-causing agents and poisons are bio activated by CYP2E1, involving the catalyst in the beginning of hepatotoxicity brought about by specific classes of medications (9). CYP2E1 also carries out the metabolism of endogenous fatty acids such as the ω-1 hydroxylation of fatty acids such as arachidonic acid, involving it in important signaling pathways that may link it to diabetes and obesity. Thus, it acts as a mono-oxygenase to metabolize arachidonic acid to 19-hydroxyeicosatetraenoic acid (19-HETE) (10). The aim of this study to assessment the cytochrome P450 gene expression changes of three variants, CYP1A2, CYP2B6, and CYP2E1 in Iraqi patients with chronic liver disease (CLD), (hepatitis + alcoholism and hepatitis + non-alcoholism).

Materials and Method

1- Subjects of the study:

The samples of fresh blood were collected in GIT Centre in Merjan teaching hospital from 50 patients with CLD and 50 healthy subjects as control group. The aged and gender of both groups were matched (p-value > 0.05).

2- Methods: Gene Expression Analysis of CYP1A2, CYP2B6, and CYP2E1

Total RNA was extracted from fresh blood of patients and control by using the TRIzol reagent (USA). The concentration of total RNA was measured by spectrophotometry and the OD260/OD280 ratio was obtained to assess the RNA purity. cDNA synthesis performed by reserve transcript and conducted by PrimeScriptTM RT-PCT reagent Kit (Korea) in a 50 μL reaction mixture following the supplier’s instructions. The qRT-PCR was performed by using cDNA as a template in the Exicyclere Real-Time PCR System (Bioneer, Korea) with SYBR green kit as fluorescent dye according to the protocol of manufacture. The PCR conditions were 95 °C for 1 min, followed by 40 cycles of 95 °C 15 s, 62 °C 45 s. The primers used in the RT-PCR were designed by NCBI tool and not showing here. The relative gene expression levels were calculated on the basis of 2−ΔΔCt. GAPDH was conceder as housekeeping gene as control. The results are presented as fold change of CYPs in patients group compare to control (GEF).

3- FAS levels estimation: Assessed by ELISA depending on Elabscience™ protocol by manufacturer instructions provided with kit.

4- Statistical analysis: Data have been analyzed statistically using SPSS program version 21. Analysis of quantitative data was done using t-test and ANOVA analysis.

Results

Clinic-pathological characteristics of patients with CLD included in this study are illustrate in table 1:

Table (1): Clinic-pathological characteristics of patients included in this study

<table>
<thead>
<tr>
<th>Clinic-pathological variables</th>
<th>NO.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total No. of patients</td>
<td>50</td>
<td>100%</td>
</tr>
<tr>
<td>* Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- &lt;30</td>
<td>22</td>
<td>44%</td>
</tr>
<tr>
<td>- ≥30</td>
<td>28</td>
<td>54%</td>
</tr>
<tr>
<td>* Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Male</td>
<td>26</td>
<td>52%</td>
</tr>
<tr>
<td>- Female</td>
<td>24</td>
<td>48%</td>
</tr>
<tr>
<td>*Hepatitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Hepatitis + Alcoholism (HC)</td>
<td>20</td>
<td>40%</td>
</tr>
<tr>
<td>- Hepatitis + Non-Alcoholism (HNC)</td>
<td>30</td>
<td>60%</td>
</tr>
</tbody>
</table>

Figure 1, showing the mean±sd of levels of FAS (ng/ml) in patients study group compare to control:
From the above figure, there were statistically significant differences in FAS levels (ng/ml) between study groups, HC, HNC, and control (p-value<0.05).

Table 2, showing the levels of FAS (ng/ml) in patients study group depending on age and gender compare to control.

**Table (2): Mean values of FAS levels (ng/ml) for patients compared with control group.**

<table>
<thead>
<tr>
<th>Clinic-pathological variables</th>
<th>FAS (mean± SD) ng/ml</th>
<th>FAS (mean± SD) ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age</td>
<td>Gender</td>
</tr>
<tr>
<td></td>
<td>≥ 30</td>
<td>&lt; 30</td>
</tr>
<tr>
<td>CLD (Hepatitis)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Hepatitis + Alcoholism (HC)</td>
<td>123.7±11</td>
<td>113±12</td>
</tr>
<tr>
<td>-Hepatitis +Non- Alcoholism (HNC)</td>
<td>67.9±3</td>
<td>62.3±3</td>
</tr>
</tbody>
</table>

From the above table, there were statistical significant differences in levels of FAS ng/ml in HC and HNC subgroups ≥ 30 and < 30 (p-value< 0.05) and this mean that age was risk factor for HC and HNC but the gender in HC was no statistical differences (p-value>0.05) while in HNC the gender was risk factor and increased in males compare to females.

The cytochrome P450 gene expression changes fold of three variants, CYP1A2, CYP2B6, and CYP2E1 in Iraqi patients with chronic liver disease (CLD), (hepatitis +alcoholism and hepatitis +non-alcoholism) compare to control group were illustrated in figure 2:
Discussion

The present study examined the fold of major drug metabolizing P450 (CYP1A2, CYP2B6, and CYP2E1) gene expression fold GEF and FAS levels in 50 Iraqi chronic liver disease (CLD) (hepatitis +alcoholism and hepatitis +non-alcoholism), and compared with 50 normal subjects. FAS is a multi-enzyme protein that catalyzes synthesis of fatty acid. FAS is not a single enzyme but a whole enzymatic system composed of two identical 272 kDa multifunctional polypeptides, in which substrates are handed from one functional domain to the next (11). In the present study, the serum FAS levels of the HC patients were found to be significantly higher in comparison to the HNC and healthy controls, indicating that high concentrations of FAS in serum may result from enzyme secretion by abnormal liver cells. Dorn C et al (2010) were reported that the transcriptional induction of FAS expression in hepatic steatosis is impaired in nonalcoholic steatohepatitis, while hepatic inflammation in the absence of steatosis does not affect FAS expression, suggesting that FAS may be serve as a new diagnostic marker or therapeutic target for the progression of nonalcoholic fatty liver disease (12). Li M et al (2018) were suggested that the accumulation of free fatty acids in hepatocytes induces lipotoxicity, leading to non-alcoholic fatty liver disease (13). GEF in CYP1A2 gene showing no change between HC and control and there were no statistical variations in GEF in HNC and both HC or control, this means that CYP1A2 was not involved in progression of HC and HNC to promoting of chronic liver disease. CYP2B6, and CYP2E1 genes were found help to progression of HC and HNC of chronic liver disease because of highly alteration in GEF in different these groups and this agreement with other studies that using genetic markers as diagnostic agents(14,15). These results suggest a significant role of CYP2B6, and CYP2E1 genes in the regulation of hepatic lipid metabolism via the fatty acid synthesis pathway and FAS, a critical factor for lipid synthesis. In conclusion, The identification of effective gene expression of CYP2B6, and CYP2E1 genes and FAS as molecular and bio markers of HC and HNC could improve the early detection of CLD.

Conflicts of Interest: No conflicts of interest to declare in relation to this work.

Acknowledgment: The authors thanks all the staff of GIT Centre in Merjan teaching hospital for contribution.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

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The Reliability of Orthodontic Treatment, According to the Needs of Patients Using the Dental Aesthetic Index

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Abstract

Objectives: Malocclusion was and remains one of the most common problems which affects the psyche and social status of the individual, so the estimation of the malocclusion severity and needs a percentage of orthodontic treatment of Iraqi patients is the aim of this study.

Method: A randomly selected 150 pairs of study models (48 male and 102 female) were involved in this study for patients attending an orthodontic clinic at College of Dentistry/ University of Baghdad seeking for treatment. The DAI scores were collected according to WHO guidelines directly from the study model with a digital caliper, score was calculated using the regression equation of 10 occlusal traits. The dental casts were classified into four groups to determine the treatment needs. SPSS software version 25 was used to analyze the results.

Results: 8.7% of orthodontic treated patients were with normal or mild malocclusion (DAI≤25) and did not need treatment, while 56% of them were handicapped and needed mandatory treatment. In between them, patients with definite and severe malocclusion were 18.7% and 16.6% respectively. Molar deviation (72.6%) represents a high prevalence rate among occlusal traits, while negative overjet (7.3%) is the least one.

Conclusions: Not all patients who are treated in an orthodontic clinic are really in need, and it must be the implementation of treatment need index to determine the treatment priority for patients.

Keywords: Dental aesthetic index, Treatment need, Study model, Orthodontic patient.

Introduction

Malocclusion is and remains one of the most common problems which affects the psyche and social status of individual. In addition, a good appearance of the teeth improves acceptance among peer group and increase successful life outcomes in comparison with people of less attractiveness.¹,²,³ One of the major problems in the evaluation of malocclusion is the presence of suitable and objective method to assess and record the severity, complexity, prevalence of malocclusion and the treatment needs.⁴ For that this reason many orthodontic indices have been developed since for decades.⁵

William Shaw and colleagues in 1995 classified occlusal indices into five groups;⁶ Diagnostic indices, Epidemiologic indices, Orthodontic treatment need indices, Orthodontic Treatment Outcome indices and Orthodontic Treatment Complexity Indices.⁷ The Dental Aesthetic Index (DAI) is one of treatment need indices, developed by Cons et al (1986, USA), It has been adopted as a cross-cultural index by the World Health Organization (WHO), a number of researches revealed that the DAI is valid and reliable.⁸,⁹ The index show single score which combine subjective, objective and clinical esthetic factors with a threshold limit (i.e. 31 or higher) to regularize with the needs for orthodontic treatment according to the severity of malocclusion.¹⁰,¹¹ It has been used in several researches within different countries in clinical and epidemiological studies of malocclusion.¹²

The purpose of this study was to estimate severity of malocclusion and needs percentage for orthodontic treatment of Iraqi patients who seek treatment in orthodontic clinic using DAI to know whether they have serious orthodontic problems or not.
Method

Data for this project were retrospectively collected from the orthodontic clinic at College of Dentistry/ University of Baghdad for attending patients who received treatment during the period of September 2017 to January 2019.

A randomly selected 150 pairs of study models (48 male and 102 female) were involved in this study. The patients age ranged from 18 to 25 years with no previous orthodontic treatment, cleft lip and palate, great restorations/crown and/or prosthetic treatment.

The DAI scores were collected according to WHO guidelines directly from the study model with a digital caliper; the index consists of 10 occlusal characteristics including; visible tooth loss, crowding in the incisor region, spacing in the region of incisors, diastema, anterior maxillary misalignment, anterior mandibular misalignment, anterior maxillary overjet, anterior mandibular overjet, vertical anterior open bite and anteroposterior molar relationship.

The core was calculated using the regression equation of 10 occlusal traits: “(visible missing teeth x 6) + (crowding) + (spacing) + (diastema x 3) + (anterior maxillary misalignment) + (anterior mandibular misalignment) + (anterior maxillary overjet x 4) + (anterior mandibular overjet x 4) + (anterior vertical open bite x 4) + (anterioposterior molar relationship x 3) + 13”.14

Then the dental casts were classified into four groups to determine the treatment needs: Those with score of ≤25 were considered as normal or mild malocclusion with little or no need for treatment, scores of 26-30 were defined as malocclusion with elective need for treatment, 31-35 were considered as severe malocclusion with highly desirable need for treatment and if the score ≥36 then it was considered as very severe or disabling malocclusion with mandatory treatment.15,16

Statistical analyses. The results were analyzed using SPSS software version 25. The statistics will be:

1) Descriptive statistics: including frequency and percentage.

2) Chi square: To test genders differences.

Calibration: To estimate the reproducibility and validity of the research, 20 dental casts were examined by a specialist orthodontist, and re-examined by the same orthodontist with an interval of 2 weeks to realize intra-examiner accuracy in the employment of the DAI. The intrarater correlation coefficient for repeated examinations was 0.96 (P < 0.001), indicating high accuracy.

Results

From the hundreds of patients seeking treatment, who visited orthodontic clinic at college of dentistry every year, 150 pairs of dental casts for pretreated patients have been used for this study. Chi-Square test showed no significant difference between male and female at a p-value (0.412) as shown in table 1.

The DAI scores were explained in Table 1 and 2, 8.7% of casts with normal or mild malocclusion (DAI≤25), 18.7% of casts with definite malocclusion (DAI 26-30), 16.6% of casts with severe malocclusion (DAI 31-35) and finally 56% of casts with handicap malocclusion (DAI ≥35). The distribution of the total sample according to their DAI scores is illustrated in Table 2, the lowest DAI score recorded was 18 (0.67%) while the highest DAI score registered was 91 (0.7%) and the most commonly recorded DAI score was 34 (7.3%).

The distribution of malocclusion components according to the DAI show molar deviation, crowding and anterior maxillary misalignment is the most common among patients which represent 72.6%, 68.7% and 66.7% respectively, and the negative overjet is the lowest among patients (7.3%) as explained in Table 3 and 4.
Table 1 Malocclusion evaluation according to Dental Aesthetic Index. And Gender differences

<table>
<thead>
<tr>
<th>Dental Aesthetic Index</th>
<th>Female n (%)</th>
<th>Male n (%)</th>
<th>Total n (%)</th>
<th>Malocclusion Severity</th>
<th>Treatment requisite</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤25</td>
<td>10 (6.6%)</td>
<td>3 (2%)</td>
<td>13 (8.7%)</td>
<td>Normal or mild occlusion</td>
<td>Little or no need</td>
</tr>
<tr>
<td>26-30</td>
<td>18 (12%)</td>
<td>10 (6.6%)</td>
<td>28 (18.7%)</td>
<td>Defined malocclusion</td>
<td>Elective</td>
</tr>
<tr>
<td>31-35</td>
<td>17 (11.3%)</td>
<td>8 (5.3%)</td>
<td>25 (16.6%)</td>
<td>Severe malocclusion</td>
<td>Highly desirable</td>
</tr>
<tr>
<td>≥35</td>
<td>57 (38%)</td>
<td>27 (18%)</td>
<td>84 (56.2%)</td>
<td>Very severe or disabling malocclusion</td>
<td>Mandatory</td>
</tr>
<tr>
<td>Total</td>
<td>102 (68%)</td>
<td>48 (32%)</td>
<td>150 (100%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Chi square (X²) 46.449  
P= 0.412

Table 2 Distribution of the total sample according to their DAI scores

<table>
<thead>
<tr>
<th>DAI Grade</th>
<th>DAI</th>
<th>n</th>
<th>%</th>
<th>Cumulative %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal or mild occlusion</td>
<td>18</td>
<td>2</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>Defined malocclusion</td>
<td>20</td>
<td>1</td>
<td>0.7</td>
<td>2</td>
</tr>
<tr>
<td>Severe malocclusion</td>
<td>22</td>
<td>1</td>
<td>0.7</td>
<td>2.7</td>
</tr>
<tr>
<td>Very severe or disabling malocclusion</td>
<td>23</td>
<td>2</td>
<td>1.3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>3</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>4</td>
<td>2.7</td>
<td>8.7</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>5</td>
<td>3.3</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>2</td>
<td>1.3</td>
<td>13.3</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>4</td>
<td>2.7</td>
<td>16</td>
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<td></td>
<td>29</td>
<td>7</td>
<td>4.7</td>
<td>20.7</td>
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<td>10</td>
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<td>29.3</td>
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<td>33</td>
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<td>1.3</td>
<td>32.7</td>
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<td></td>
<td>34</td>
<td>11</td>
<td>7.3</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>6</td>
<td>4</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>8</td>
<td>5.3</td>
<td>49.3</td>
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<td>37</td>
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<td>1.3</td>
<td>50.7</td>
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<td>38</td>
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<td>4.7</td>
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<td>57.3</td>
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<td></td>
<td>40</td>
<td>4</td>
<td>2.7</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>41</td>
<td>4</td>
<td>2.7</td>
<td>62.7</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>4</td>
<td>2.7</td>
<td>65.3</td>
</tr>
</tbody>
</table>
Table 3 Distribution of dentition, occlusion and space components according to DAI

<table>
<thead>
<tr>
<th>DAI components</th>
<th>Present n</th>
<th>%</th>
<th>Absent n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dentitions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tooth loss</td>
<td>30</td>
<td>20</td>
<td>120</td>
<td>80</td>
</tr>
<tr>
<td>One tooth</td>
<td>20</td>
<td>13.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Two tooth</td>
<td>9</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Three tooth</td>
<td>1</td>
<td>0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Space</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crowding</td>
<td>103</td>
<td>68.7</td>
<td>47</td>
<td>31.3</td>
</tr>
<tr>
<td>Single jaw</td>
<td>46</td>
<td>30.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Both jaw</td>
<td>57</td>
<td>38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spacing</td>
<td>54</td>
<td>36</td>
<td>96</td>
<td>64</td>
</tr>
<tr>
<td>Single jaw</td>
<td>31</td>
<td>20.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Both jaw</td>
<td>23</td>
<td>15.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median Diastema</td>
<td>29</td>
<td>19.3</td>
<td>121</td>
<td>80.7</td>
</tr>
<tr>
<td>Anterior Maxillary Misalignment</td>
<td>100</td>
<td>66.7</td>
<td>50</td>
<td>33.3</td>
</tr>
<tr>
<td>1-3 mm</td>
<td>66</td>
<td>44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-6 mm</td>
<td>32</td>
<td>21.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥7mm</td>
<td>2</td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior Mandibular Misalignment</td>
<td>81</td>
<td>54</td>
<td>69</td>
<td>46</td>
</tr>
<tr>
<td>1-3 mm</td>
<td>72</td>
<td>48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-6 mm</td>
<td>9</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥7mm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Occlusion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overjet (&gt; 4mm)</td>
<td>67</td>
<td>44.7</td>
<td>83</td>
<td>55.3</td>
</tr>
<tr>
<td>Negative overjet</td>
<td>11</td>
<td>7.3</td>
<td>139</td>
<td>92.7</td>
</tr>
<tr>
<td>Anterior open bite</td>
<td>21</td>
<td>14</td>
<td>129</td>
<td>86</td>
</tr>
</tbody>
</table>

Table 4 Distribution of molar relationship component according to the DAI

<table>
<thead>
<tr>
<th>Molar relationship</th>
<th>Present</th>
<th>Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Percent%</td>
</tr>
<tr>
<td>Deviation from the normal molar relationship</td>
<td>109</td>
<td>72.6</td>
</tr>
<tr>
<td>Half cusp</td>
<td>62</td>
<td>41.3</td>
</tr>
<tr>
<td>One cusp</td>
<td>47</td>
<td>31.3</td>
</tr>
</tbody>
</table>
Discussion

The orthodontic clinic at the college of dentistry receives a large number of patients annually from across the country due to lack of specialist orthodontists and the cost of treatment.

In this research, measurements were performed on studying models instead of patients in order to examine a wide variety of patients within a short time, in addition to knowing the patients who visit the orthodontics clinic, the eligible ones for treatment.

The DAI has been supported by WHO due to its simplicity, reliability and wide use in researches, in addition to its high sensitivity in predicting the high proportion of persons requiring orthodontic treatment correctly as well the no-treatment need.

The components of the DAI show the percentage of malocclusion traits and those results can highlight the most commonly presented one as explained in Table 3.

Only one Iraqi study of Kurdish people and few studies of other populations have DAI analysis of malocclusion traits. Also, because this study is the first study carried out for orthodontic patients who attend the orthodontic department so the results were completely different from previous Iraqi studies. Besides the differences in genetics and race, lead to differences from other populations.

Concerning orthodontic treatment needs, 8.7% of treated patients represent mild or normal occlusion with little or no need for treatment (DAI≤25), while 16.6% (DAI 31-35) of patients have severe malocclusion and 56.2% (DAI≥35) are considered as being handicapped and in need of mandatory treatment. The female represents 68% of the total sample and that indicate the concern of female about their occlusion and appearance more than male.

Regarding the handicapped malocclusion, the results of our study is close to the results of Poonacha et al. (55%) and Goyal et al. (51%); Also, because this study is the first study carried out for orthodontic patients who attend the orthodontic department so the results were completely different from previous Iraqi studies. Besides the differences in genetics and race, lead to differences from other populations.

But what attracts attention is that orthodontic patients with (DAI≤25) are clearly different from those of other studies which depends on patients who visited the clinic in general. While, our study uses the study models of previously treated patients.

The percentage of each parameter of DAI is explained in Table 3. Where tooth loss was present in 20% of total samples, Crowding in 68.7%, spacing in 36%, median diastema 19.3%, anterior maxillary misalignment 66.7%, anterior mandibular misalignment 54%, overjet (> 4mm) 44.7%, negative overjet 7.3%, anterior openbite 14% and molar deviation from the normal relationship 72.6%.

As we have clarified, the result of each anomaly show much higher rate in comparison with other studies because it harmonious with the fact that the sample was composed entirely of patients referred to an orthodontics clinic with malocclusion, in addition to the racial, genetic, social behaviors, cultural differences and the most common effects are economic and political reasons.

Conclusion

Not all treated patients in the orthodontic clinic are with a true need for treatments, but few of them are with mild malocclusion and no need for treatment; since the therapeutic possibilities are minimally available and poor economic situation for most of the people, the treatment needs should be provided for patients who really deserve.

The DAI is the simplest and easiest index to be used by a general dentist for examination of attending to patients and assessing the severity of their malocclusion to be treated as a priority treatment need.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References


Study the Protective Role of Arabic Gum Extract on Some Physiological and Histological Criteria for Liver of Male Rabbit Treated with Atrazine

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¹Department of Biology, College of Education for pure Sciences, Karbala University, Iraq

Abstract
The study involved fifteen health adult male rabbit white (*Lepus arcticus*), weight ranged (1480-1550) kg. and age between 6 to 8 month, divided to three groups the first group treated 10 ml of atrazine orally for five rabbits daily for two weeks and second group is treated with aqueous extract of Arabic gum (AG) (*Acacia Senegal*) 30% and atrazine 10 ml between one day and another for two weeks and third group was the control group given 10 a normal saline. In the search the levels of hepatic enzyme were presented (AST) Aspartate transaminase, (ALT) Alanine transaminase and (ALP) Alkaline phosphate, also the rate of diameters of liver sinuses, the diameters of central veins and hepatocellular diameter, and found through this study:

- The presence of a significant increase (P≤0.05) in the levels of hepatic enzyme (AST), (ALT) and (ALP) and rate of diameters each of liver sinuses, central veins, and hepatic cells in atrazine group compared with the control group.

- The presence of significant decrease (P≤0.05) in levels of hepatic enzyme (AST) and (ALT) in the group treated with aqueous extract of AG in 30% concentration and 10 ml of atrazine compared with control group, and did not occur significant differences (P≥0.05) in the level of (ALP) and in rate of diameters each of liver sinuses, central veins, and hepatic cells in aqueous extract of *Acacia Senegal* and atrazine group compared with the control group.

**Keyword:** Atrazine, Acacia Senegal, AST, ALT, ALP, Hepatocytes.

Introduction
Medicinal plants containing active substances used for therapeutic purposes or in the pharmaceutical industry and that the natural products of these plants are an effective source to discover vital effectiveness like anticancer, antioxidant (¹), antibacterial and antiparasitic (²). Where these plants are a food source on one hand and medication against various diseases on the other due to the contents of its parts of chemical compounds of great interest and importance because of therapeutic activity of humans (³), where used as catalysts for growth (⁴), the orientation for medicinal plant treatment is justified and taking chemically manufactured drugs has been shown to have serious side effects that may appear over time and cumulatively (⁵).

AG is a substance collected from the secretion of the stems and branched of *Acacia Senegal* tree, it consists mainly of large molecules carbohydrates, proteins, minerals and amino acid (⁶). AG has a high molecular weight as polysaccharide it contains galactose, rhamnose, glucuronic acid and arabinose also calcium, potassium and sodium salts. It also contains 1.5%-2.6% protein, 0.22%-0.39% nitrogen and amino acid mainly like hydroxyproline, serine, proline, and aspartic acid, it has high solubility in water but does not dissolve in alcohol (⁷). And has many uses in the industrial section such as textiles and cosmetics, and pharmaceutical and nutritional uses where in the pharmaceutical field as a pharmaceutical compound because of its antioxidant activity and positive effect in treatment of urinary tract, cardiovascular and digestive system also improves the liver, kidney & heart tissue (⁷).
Atrazine effects on the body’s various organs, including the liver, which is the main organ for detoxification of the body. Research has shown a decrease in the accumulation of hepatic glycogen and the presence of early symptoms of liver cytotoxicity, this is due to the toxic effects of atrazine on the liver that inhibit the activity of major enzymes such as hexokinase, glycogen synthase and glucokinase(8); this explains the low body weight has been observed to lead to decrease in glycogen and increase fat in liver(9). In addition, bile duct hypertrophy and renal tube necrosis have been observed (10). It has also been found to lead to changes in insulin resistance and disturbance in fat digestion (11). Atrazine also attacks fats, proteins and DNA molecules, causing metabolic changes in severe cases, it can lead to cell death and lead to oxidative stress and emergence and development of many diseases such as atherosclerosis, cancer, psoriasis, Alzheimer’s, high blood pressure, heart disease, liver, kidney and brain (12).

Materials and Method

AG samples were collected from Babylon governorate in October 2018, Gum Arabic powder was moistened with water by 1:5 where 50 of gum Arabic was mixed with 250 ml of distilled water (13). a horizontal shaker GFL type, 3015 modules for 30 minutes and the sample was left static and then filtered using filter paper three times, after that centrifuge was used 3000 rpm for 15 minutes. the extract was concentrated using a rotary evaporator and dried 45 in an oven.

Experimental design.

The experiment was designed to investigate the effect of aqueous extract of AG and atrazine on liver tissue and its effect on some functional blood parameters in local male rabbits. then divided into three groups, including 5 rabbits are:

1- Atrazine group 10 ml for two weeks daily.

2- Aqueous extract of AG and atrazine group from day to day at dose of 10 ml and at concentration of 30% of AG and 10 ml of atrazine and for two weeks daily.

Anatomy of animals and blood collection

The animals were then numbed with chloroform and anatomy by opening the abdominal cavity and draw blood through heart puncture to get the most amount of blood. Blood samples were placed directly into sterile anticoagulant –free test tubes its capacity 10 ml, it was left for 15-20 minutes at laboratory temperature and then transferred to a centrifuge 3000 rpm for 15 minutes for the purpose of obtaining the serum stored in the refrigerator at temperature -4 C(15)for the purpose of physiological testing that including AST and ALT (16) ALP(17). The liver was removed after fatty substances were removed fixed with formalin concentration 10% so as to preserve the cellular structure and the natural structure and the natural state of the tissue, after two days 48 hours I was extracted from formalin and washed with tap water for a (3-6) hours, then a series of operation took place to prepare tissue slides colored Eosin and Hematoxylin then the prepared tissue slides were examined using a compound microscope and under a 10X magnification force, and measured the rate of diameters of liver sinuses, the diameters of central veins and hepatocellular diameter.

Results and Discussion

Physiological study: The results of the physiological study are shown in Table 1 for atrazine group 10 ml in rabbits’ serum there is a significant increase (P ≤ 0.05) in the average level of ALT, AST and ALP enzymes for two weeks compared with the control group. The results of this study are consistent with which reached Jested and his group (18) in his study with doses rats 300mg/kg from atrazine for one month noting an increase in the levels enzymes mentioned above , consistent with the results of this study of Hussein and his group (19) which he made on rats by taking them 400gm/kg from atrazine for two weeks ,the cause of the increase in rate of enzymes levels in the blood serum indicates the extent of damage to the liver and heart tissues the result of treatment with atrazine leading to a change in hepatic metabolic functions through damage to hepatocytes or the increase may due to kidney damage due to heart damage which leads to the occurrence of chronic renal insufficiency due to necrosis, degeneration or damage to renal cells leading to enzymes leaking into the blood serum (20) Or due to increased oxidative stress resulting from the high proportion of oxidant from the liver because atrazine is an oxidizing agent capable of attacking the cell membrane and antioxidant molecules which cause damage and breakdown in DNA , protein and fat(21).consistent with this result what he pointed out Mohammad and his group (22) .to born of free radicals (ROS) Reactive Oxygen Species leads to the breakdown of mitochondria containing the enzymes listed, because oxidative stress increases the process of lipid peroxidation leading to cirrhosis.
The results of the physiological study are shown in Table 1 for atrazine group 10 ml that treatment with aqueous extract of AG at concentration in rabbits to presence of significant decrease (P≤0.05) in AST and ALT, there is no significant difference (P≥0.05) in rate of ALP for two weeks compared with the control group. Consistent with the results of our study (23), noting when dosing mice with 10% from AG for three month leads to decrease in the concentration of enzymes mentioned above, the results of the atrazine group with the results of this group treatment with AG to increase in average level of liver cell enzymes that lead to oxidative stress of the kidney and liver that results born of free radicals capable of attacking the cell membrane and antioxidant molecules which cause damage and breakdown in DNA, protein and fat (22). Also the study of Ayaz and his group (24) referred to the protective role of aqueous extract go AG when give 0.5 g/kg daily for two weeks to mice treated with toxic substance Trichloroactate where they notice a decrease in the levels of hepatic enzymes mentioned above perhaps due reason to improvement in the liver functions due to increased enzymatic antioxidants such as Catalase and Glutathione peroxidase, and non-enzymatic such as vitamin A,C, and because the plant contains phenolic compounds which working on decrease free radical formation (25).

Table (1): effects of aqueous extract of AG in concentration 30% on levels rate of some enzymes in serum of rabbits that treated with atrazine.

<table>
<thead>
<tr>
<th>parameters groups</th>
<th>AST U/L</th>
<th>ALT U/L</th>
<th>ALP U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>18.80± 0.84</td>
<td>20.60± 1.52</td>
<td>23.40± 3.78</td>
</tr>
<tr>
<td>Normal saline (10)ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrazine 10 ml</td>
<td>27.38± 3.91</td>
<td>29.40± 1.52</td>
<td>44.60± 6.50</td>
</tr>
<tr>
<td>Atrazine 10 ml and gum Arabic 30%</td>
<td>16.30± 0.84</td>
<td>18.80± 0.84</td>
<td>23.46± 2.21</td>
</tr>
<tr>
<td>LSD</td>
<td>2.74</td>
<td>1.51</td>
<td>4.89</td>
</tr>
</tbody>
</table>

**Histological study:** Figure 1 represents the normal histological structure of the control group for two weeks for a rabbit liver, the liver consists of a central vein and hepatic cords surrounding cubic cells distributed between hepatic cord and hepatic sinuses. The results have shown the formal and histological measurements in Table 2 and Figure 2 to section of liver tissue for atrazine group treated with AG 30% to absence of significant differences (P≥0.05) in diameters rate each of hepatic sinuses, central vein and hepatic cells compared with the control group, as for the histological composition for atrazine group treated with AG 30% note a slight improvement in liver tissue represented by lack of central vein expansion compared with group treated with atrazine alone, absence lack in sinuses and less cellular infiltration.

The study of (26) pointed to that oral dosage with alcoholic extract of AG plant 400,800mg/kg daily for one week for mice treated with toxic carbon tetrachloride prevent necrosis, fatty infiltration and liver damage resulting from this toxic substance CCL4 that the histological structure of the hepatic cords and tissue normal appears addition to low in hepatic enzymes. This confirms the effectiveness of AG as a medicinal plant used in folk medicine because it contains many effective chemical compounds such as carbohydrates rhamnose, galactose and arabinose, acids such as glucuronic and mineral salts such as calcium, potassium, magnesium and sodium, addition to medically effective compounds such as phenols, flavonoids and alkaloids (27). Also the study of Ram and his group pointed to a role of alcoholic extract of gum Arabic plant when dosing rabbit 500mg/kg daily for forty five days leads to remain the liver, kidney, heart and aorta tissues similar to normal, addition to low in hepatic enzymes and liver lipids that pointed to the possibility of the plant in the treatment of cases of
fats and atherosclerosis\(^{(28)}\).

Table (2): Measurement of liver sinusoids diameters, central veins diameters and hepatocyte diameters measured in micrometers for male rabbits after dosing with aqueous extract of AG and atrazine for two weeks.

<table>
<thead>
<tr>
<th>hepatocytes groups</th>
<th>Diameters of liver sinuses µM</th>
<th>Diameters of central veins µM</th>
<th>Diameters of hepatic cells µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group Normal saline (10)ml</td>
<td>41.2 ± 9.1</td>
<td>72.7 ± 8.4</td>
<td>17.3 ± 2.0</td>
</tr>
<tr>
<td>Atrazine 10 ml</td>
<td>80.7 ± 2.3</td>
<td>*98.7± 209</td>
<td>*23.2 ± 3.1</td>
</tr>
<tr>
<td>Atrazine 10 ml and AG 30%</td>
<td>46.9 ± 9.1</td>
<td>80.2 ± 9.0</td>
<td>19.9 ± 3.1</td>
</tr>
<tr>
<td>LSD</td>
<td>13.0</td>
<td>12.2</td>
<td>2.9</td>
</tr>
</tbody>
</table>

Figure 1 Section of liver tissue in control group for two weeks notes presence of the central vein (➜) regularity in the hepatic cords (➜) with presence of sinusoids (➜) (10X H&E)
Figure 2: Section of liver tissue in atrazine group treated with 10 ml for two weeks notes presence of congestion and dilatation of central vein (➜) and portal vein (➜) dilatation in sinusoids (➜) cellular infiltration (➜) severe irregularity in hepatic cords (➜) with water degeneration (➜). (10X H&E)

Figure 3: Section of liver tissue in atrazine group treated with 10 ml for two weeks with AG with 30% concentration notes presence of congestion and dilatation of central vein less than previous treatment (➜) less cellular infiltration (➜) presence water degeneration (➜) dilatation in sinusoids (➜). (10X H&E)
Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References


Relation between Working in Petrol Station and Blood Hemoglobin Levels for the Filling Workers in Al-Najaf City/ Iraq

Fulath Abdul-Redah Muhsin
Community Health/The Head of Community Health Department / Al-Kufa Technical Institute / Al-Furat Al-Awsat Technical University

Abstract
Petrol station workers were exposed to several pollutants like air pollutants by inhalation, or skin contact with petroleum derivatives like benzene, and another port of entry. This comparative cross-sectional study was conducted to find a relationship between working in petrol stations and hemoglobin blood levels of the workers in Al- Najaf-city. The exposed worker’s group was (50 male) of petrol station workers aged 20-50 years, the years of working was (1 ≥15 years) of ( ≥7 h/day) that included in this study, the comparison group was 50 healthy male service and office workers aged (20-50 years) matching with the study group from al-Kufa Technical Institute, Al-Furat Al-Awsat Technical University.

Blood samples were tested at the field by using a digitized portable device (hemochromax plus) which gave the blood hemoglobin concentrations at mg/dl of the petrol stations worker. The results of blood hemoglobin were compared between both groups. There was a highly statistically significant difference (HS) between Gas station workers and Control group at the $P$-value of (0.001) of Hb blood concentration levels and the mean of gas station worker and control group were (11.57 and 15.58 mg/dl) respectively. there was a highly statistically significant difference (HS) between not anemic and anemic workers at the $P$-value (0.000), and there were 47 workers (94%) out of all 50 workers had anemia after the field test.

There was a statistically significant difference (HS) between Hb levels of Gas station workers and Standard Normal Hb Level at the $P$-value = 0.007. There were a moderate inverse negative correlated between the time of exposure in the year and blood hemoglobin levels in mg/dl at the ($r = -0.57$), which mean there were decreases of blood hemoglobin levels of the worker when increases of the duration of service employment. The study concluded from all results that were finding that decreased blood hemoglobin concentration than the normal value of the petrol stations workers for all stations, which might be due to the adverse effect of the workplace pollutant on bone marrow. Attention should be given by a periodical medical assessment of all workers in the petrol stations, and Obligate the petrol stations managers for supplies all personal protective equipment for all workers.

**Keyword:** workplace pollutants, Hemoglobin, Anemia, Filling Workers, Al- Najaf city.

Introduction
Health impact of occupational exposure to petrol and air pollution from the exhaust of automobile sources and un discover through petrol station workers (1). Lack of ventilation &unused of the personal protective device at the work place when using benzene will arise the occurrence of Toxic effects of benzene and petrol derivatives in workers (2). Repeated exposure can lead to inflammation of the respiratory tract and hemorrhage in the lung. Different air pollution like benzene and atmospheric polluted air like car exhausts, absorbed into the human body by respiratory tract or via epidermal contact (3).
Air pollutants and other chemicals like benzene or other heavy metals and carbon monoxide (CO) and carbon dioxide (CO$_2$) can cause adverse health effects by body metabolites and interference with biochemical or physiological processes of the human body (4). Petroleum derivatives were used for different reasons by human beings at home, in manufacturing and petrol station (5).

Petrol station workers are exposed to a mixture of hydrocarbons in a fuel vapor through dispensing fuel and to the gases from car exhaust (6). In the petrol station; the amount of fuel spread as well as the ambient temperature interaction significantly to the arising emission of volatile hydrocarbons. Most people have a greater risk of exposure to gasoline vapors, these include petrol station workers, service station and drivers of cars (7).

The nature of the petrol station workers makes them readily available of the most time to exposed by skin or ingestion and inhalation. Benzene & other derivatives affect blood production by affecting the bone marrow. the most characteristic effect resulting from intermediate and chronic benzene exposure was reduced the development of blood cell (8).

And causes aplastic anemia in human. The clinical finding in petrol. Hepatotoxicity cytopenia; which was a decrease in several cellular elements of circulating blood as manifested as anemia; leukopenia and thrombocytopenia in humans. The inhalation of petrol derivatives like benzene vapor is rapidly absorbed into the blood and distributed through the body. Several studies of benzene-exposed workers agreed that chronic exposure to benzene at air resulting in the adverse hematological effects (9).

The aim of this study: To find a relationship between exposures to workplace pollutants and hemoglobin blood levels of the petrol station filling workers of Al-Najaf city.

Material and Method

Study Design: Comparative cross-sectional study.

Place of the study: The study was conducted in «Al-Najaf city that located to the South of Baghdad about 165Km, Iraq».

Period of the study: Data Collection was lasted from (30/1/2018 to 19/2/2018).

Sampling collections: All petrol stations that belong to governorate in Al-Najaf city were included in this study. The blood samples were collected from 7 government gas petrol stations in Al-Najaf city, the samples were tested at field of the petrol stations. From 50 person that working on it.

Questionnaire: A well-designed questionnaire was applied in this study.

Field tests: Samples were tested by using a portable digitized device portable, on each petrol station in Al-Najaf city. was used in this study to test the Hb % level of the petrol station workers (Hemochromax plus) of Korean made which was need only one blood drop to give the Hb levels results.

Statistical methods: Descriptive and analytical statics were carried out in this study by using a statistical package from social science (SPSS) version 18. Z-test was applied to obtain only statistical significance difference and Pearson correlation coefficient (r) was used to find the correlation between exposure time in years and hemoglobin concentration in blood.

Results

Table (1): comparison between study group of petrol station workers and Comparison group among age in years.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Study group (Filling workers) n = 50</th>
<th>Comparison group n = 50</th>
<th>P-value (z-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>32.47 ± 6.7</td>
<td>34.54 ± 8.1</td>
<td>*0.47</td>
</tr>
</tbody>
</table>

* (Non-Significant)
Table (1): Show that there is non-statically significant different between age group and comparison group at P-value = 0.47.

Table (2): Comparison between study group of petrol station workers and Comparison group of Hemoglobin concentration levels.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Petrol station workers (n = 50)</th>
<th>Comparison group (n = 50)</th>
<th>P-value (z-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb concentration (mg/dl)</td>
<td>11.57 ± 1.1</td>
<td>15.58 ± 2.2</td>
<td>*0.001</td>
</tr>
</tbody>
</table>

* (Statistically Highly Significant) (HS)

Table (2): Show that there is a highly statistically significant difference (HS) between petrol station workers and Comparison group at the P-value of (0.001) of Hb blood concentration levels, and the mean of petrol station worker and the Comparison group were (11.57 and 15.58 mg/dl) respectively.

Table (3): Comparison details between normal (nonanaemic) and low concentrations (anemic) among the study group of petrol station workers of Al-Najaf city.

<table>
<thead>
<tr>
<th>Nonanemic no. (%) (workers)</th>
<th>Anemic no. (%) (workers)</th>
<th>Total No. (%)</th>
<th>P-value (z-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 (6 %)</td>
<td>47 (94%)</td>
<td>50 (100%)</td>
<td>* 0.000</td>
</tr>
</tbody>
</table>

* (Statistically Highly Significant) (HS)

Table (3): Show that there is a highly statistically significant difference (HS) between nonanemic and anemic workers at the P-value (0.000), and there were 47 workers (94%) out of all 50 workers had anemia after the field test.

Table (4): Summery statics comparison among a study group of petrol station workers Hb levels and standard normal Hb level in the adult.

<table>
<thead>
<tr>
<th>Comparison Parameter</th>
<th>Test of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>HB levels of Gas station workers × Standard Normal HB Level</td>
<td>*P - value = 0.007</td>
</tr>
</tbody>
</table>

(Based on Z-test * S)

Table (3): Show that there is a statistical significant difference (HS) between Hb levels of petrol station workers and Standard Normal Hb level at the P-value = 0.007.

(*moderate inverse negative correlated)
Figure (1): The correlation between exposure time and hemoglobin blood levels of the worker in the gas stations of AL-Najaf city

Figure (1): Show that there are a moderate inverse negative correlated between the time of exposure in the year and blood hemoglobin levels in mg/dl at the \( r = -0.57 \), which mean there are decreases in blood hemoglobin levels of the worker when increases of the duration of service employment.

**Discussion**

Air pollution and the dealing with petrol derivatives still have more priority for the worker when risk assessed, as well as its toxicological effect for the bodyworker. The study in the Al-Najaf petrol station could be considered the first study with this result finding, most of the worker of the study group were in the second decade of the life, the study group of the petrol station worker and healthy control group was matched for age to find if there is any significant difference for achieving the study aim.

All the results that we’re found in the present study between the study group of the worker and the comparison group were significantly more among the petrol station workers than the other (control group) from the normal parameter.

The present study was showed a highly statistically significant difference when comparison was made between the petrol station worker and control group in hemoglobin concentration levels, this defect might be due to the damage to the bone marrow by the toxic effect of the pollutant hydrocarbons gases that emissions from the car exhaust in the ambient air of the petrol stations and the entrance of the petroleum products like benzene or gasoline oil through the respiratory tract by inhalation or ingestion and entrance by skin contact way.

The results data in present study was showed that the most numbers and the percentage of the worker that represent of studied samples have anemia through the clear decrease of the blood hemoglobin concentration 47 (94%) respectively, with the higher statistical significant differences in comparison with non anemic worker, the same finding results were obtained by Okoro A.M. (10), of study in Nigeria of anemic worker in petrol stations at 2010.

While the different results were found in Gaza, Palestine with that mentioned by Sirddah M.M., et al in 2013, who found that the hemoglobin levels of the blood concentration levels were increases in the blood of the petrol stations workers (11).

The present study was showed a highly statically significant difference between Hb levels of petrol station workers and standard normal hemoglobin Level at the \( P\)-value = 0.007, The results agreement with that mentioned by Anthony Seaton, et al, 2016 in United Kingdom (12), who found that there was a high difference when compared with the normal Hb value. In addition, the same results were found by Tunsaringkarn T, et al, in Bangkok, at 2013, Thailand (13).

The present study showed that there was a moderate inverse negative correlated between the time of exposure and blood hemoglobin levels, \( (r = -0.57) \), which mean there was a relationship between exposure time and blood hemoglobin levels, in another meaning the decreased in blood hemoglobin levels of the filling petrol station worker was happened when increased in years of service, this results might be due to continuity in exposure to workplace pollutants, the same finding results were obtained by Sahb AA, of study in Baghdad in 2013 (14).

**Conclusions**

The following conclusions can be derived from this study:

1. There was a highly statistically significant difference (HS) between gas station workers and control group at the \( P\)-value of (0.001) of Hb blood concentration levels.

2. There was (HS) between nonanemic and anemic workers at the \( P\)-value ( 0.000).

3. There was an (HS) between Hb levels of gas station workers and standard normal Hb Level at the \( P\)-value = 0.007.

4. There were a moderate inverse negative
correlated between the time of exposure in (years) and blood hemoglobin levels in mg/dl at the \( r = -0.57 \), which mean there were decreases of blood hemoglobin levels of the worker when increases of the duration of service employment.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** Self-funding

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Effect of Preoperative Breathing Exercise on Postoperative Patients’ Lung Functions

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Abstract

Pulmonary complications after surgery are a major cause of morbidity and mortality and therefore are a source of concern in both developed and developing countries. Presently, surgical patients didn’t acquire education about deep breathing activities that were adequate and many of them only received some education post operation. Aim of the study: To investigate the Effect of Preoperative Breathing Exercise on Postoperative patients’ Lung Functions. A quantitative quasi-Experimental, Pre-Test and Post-Test design. A non-probability (purposive sample) of (60) patients divided into two equal groups distributed as the case and control groups (30) patients for the case group are exposed to the breathing exercise program, Pursed-lip method, and (30) patients without exposed to the breathing exercise program categories as control group. The study have been carried out in Al-Diwaniyah teaching hospital, This study conducted from 22nd of October 2018 to 25th of June 2019. There were statistically significant difference between case and control groups, patients in the case group had improvement in all parameters of lung functions than control group ( P < .05) in the two periods of measurement post-operatively. The program show obvious difference between the lung function for both study and control groups during the post-test.

Keywords: Preoperative, Breathing Exercise, Postoperative, patients’ Lung Functions.

Introduction

Impairment in lungs functions after surgery are one of important postoperative complications, strength of respiratory muscles could be decreased and that one reason lead to lung function change.

During surgery, using of general anesthetics can affect the central regulation of breathing by changing the neural drive to respiratory muscles such as the diaphragm, and because of this, the respiratory function cannot be separated from anesthesia. The components that can alter the pulmonary function contain unconsciousness, ventilation (whether its mechanical or spontaneous), a patient’s position, anesthetics drugs, that were used during the anesthetic process on the respiratory muscles. General anesthesia can alter mucociliary function, promoting retention of secretions, causing bronchoconstriction, decreased surfactant production, inhibition of alveolar macrophage activity. Moreover, anesthesia may cause atelectasis instantaneously in majority of the patients as an outcome of chest wall deformation, decreased inspiratory muscle tone, and reduced functional residual capacity which may considerably influence gas exchange.

The effect of these factors on pulmonary function are appear in the early postoperative period. Lung function, measured as vital capacity (VC) and forced expiratory volume in one second (FEV1), is usually decreased by 35-60%, and a 6-13% Decreased lung function can linger for four months. In the first days following an operation, a decreased lung function can impact impaired gas exchange, and the strength of the respiratory muscle will also decrease during the first days after surgery. This may be explained by insufficient diaphragmatic breathing or by the respiratory fatigue that some patients experience during the post-operative period. The complications that related to anesthesia, tissue damage, immobilization, inhibition of cough, and incision of abdominal muscles which results in postoperative pain, major causes to decreased the lung volumes and limited airway clearance, which can lead to Post-operative pulmonary complications (PPC).

Methodology

Study Design:
A quantitative quasi-Experimental, Pre-Test and Post-Test design have been carried out to study the effect of preoperative breathing exercise on postoperative patients’ lung functions, in Al-Diwaniyah teaching hospital, This study conducted from 22nd of October 2018 to 25th of June 2019.

Setting of the Study:

The study is conducted in Al-Diwaniyah City, Al-Diwaniyah Health Directorate, Al-Diwaniyah teaching hospital, Surgical Wards. This hospital is a governmental, and the largest general hospital, and presents different of the medical therapeutic services in Al-Diwaniyah city.

Study Sample:

A non-probability (purposive sample) of (70) patients undergoing abdominal surgery. (10) patients of the sample for pilot study and (60) patients of the sample is divided into two equal groups distributed as the case and control groups (30) patients for the case group are exposed to the breathing exercise program, Pursed-lip method, and (30) patients without exposed to the breathing exercise program categories as control group.

Criteria for Including the Sample:

1. Adult patients age from 20 years and above.
2. Patients undergoing abdominal surgery under general anesthesia.
3. Stable condition as reported by the physician.
4. Patients with normal respiratory and cardiac functions.

Validity of the Instrument:

The face validity of the study instruments are determined by a panel of (16) experts, have experience more than ten years, from different specialties from nursing faculties.

Reliability of Instrument:

In order obtains the reliability of the study instrument five patients undergoing abdominal surgery are selected from Surgical wards for the purpose of testing the instrument their lungs capacities (FEV1, FVC, FEV%) was measured in preoperative period by used two spirometer at one time, one of them is used in the present study and the other used at Al-Diwaniyah teaching hospital. A comparison between the results of them, The result indicate, spirometer which used in present study is a reliable instrument to measure the purpose of the study.

Through developed questionnaire for Arabic version, demographic and clinical data were collected. While physiological data (Lungs volume) are collected by using the pulmonary function test machine (Portable Diagnostic Spirometer, Mir, Italy) through preoperative and postoperative assessment. During preoperative phase, the assessment was obtained ( before one day of surgery for case group and, in the same day for control group), while in the post-operative phase, the assessment was done at 8th hours and 24th hours after surgery based on the previous scientific references such as 10 Regarding case group the assessment of the lungs volumes applied before and after the application of the program. For the control group, the assessment was performed only without the application of the program. The data collection process has been performed from 11th January to 14th March 2019.

The data have been collected through the use of a constructed questionnaire tool. The data analysis was done through statistical package of social science (SPSS) version (25). The methods of analysis of data include; Descriptive Data Analysis and Inferential Data Analysis

Results

Table (1) Clinical Data of Case and Control Groups n= 60

<table>
<thead>
<tr>
<th>Clinical Data</th>
<th>Rating And Intervals</th>
<th>Case group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Freq.</td>
<td>%</td>
<td>Freq.</td>
</tr>
<tr>
<td>Body mass index</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal weight</td>
<td>5</td>
<td>16.7</td>
<td>8</td>
</tr>
<tr>
<td>Over weight</td>
<td>13</td>
<td>43.3</td>
<td>14</td>
</tr>
<tr>
<td>Obese</td>
<td>12</td>
<td>40</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100</td>
<td>30</td>
</tr>
</tbody>
</table>
Table (1) represent the clinical data of the case and control groups. Regarding body mass index (BMI) the table shows most of the sample in both groups are overweight (46.7%) in control group while (43.3%) in case group. In smoking status condition the table shows the majority in control group (76.7%) is nonsmoker and (70%) in the case group. Regarding type of smoking in both groups are cigarette smokers with duration of smoking (66.6%) with period of (10-19) years for control group and (57.1%) for case group who spend more than 30 years in smoking. Regarding chronic disease the study results presents that chronic disease (60%) of case group with chronic disease and (66.7%) in control group with chronic disease (50%) of both group with hypertension. Regarding previous surgery (50%) in case group have previous surgery (20%) of them with Caesarian surgery.

<table>
<thead>
<tr>
<th>Smoking status</th>
<th>Nonsmoker</th>
<th>21</th>
<th>70</th>
<th>23</th>
<th>76.7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Passive smoker</td>
<td>2</td>
<td>6.7</td>
<td>1</td>
<td>3.3</td>
</tr>
<tr>
<td>Cigarette smoker</td>
<td>7</td>
<td>23.3</td>
<td>6</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100</td>
<td>30</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Duration of smoking (years)</th>
<th>10-19</th>
<th>28.6</th>
<th>4</th>
<th>66.7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20-29</td>
<td>14.3</td>
<td>1</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td>30 and more</td>
<td>57.1</td>
<td>1</td>
<td>16.7</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>100</td>
<td>6</td>
<td>100</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Chronic disease</th>
<th>Yes</th>
<th>12</th>
<th>40</th>
<th>10</th>
<th>33.3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>18</td>
<td>60</td>
<td>20</td>
<td>66.7</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100</td>
<td>30</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of chronic disease</th>
<th>Diabetic</th>
<th>3</th>
<th>25</th>
<th>3</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hypertension</td>
<td>6</td>
<td>50</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Hypertension + diabetic</td>
<td>3</td>
<td>25</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>100</td>
<td>10</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Previous surgery</th>
<th>Yes</th>
<th>15</th>
<th>50</th>
<th>13</th>
<th>43.3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>15</td>
<td>50</td>
<td>17</td>
<td>56.7</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100</td>
<td>30</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of previous surgery</th>
<th>Caesarian</th>
<th>6</th>
<th>20</th>
<th>5</th>
<th>16.7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hernia</td>
<td>2</td>
<td>6.7</td>
<td>2</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>Hysterectomy</td>
<td>1</td>
<td>3.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Appendectomy</td>
<td>3</td>
<td>10</td>
<td>1</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>Cholecystectomy</td>
<td>1</td>
<td>3.3</td>
<td>1</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>Hemorrhoidectomy</td>
<td>2</td>
<td>6.7</td>
<td>1</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>Gastric surgery</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>100</td>
<td>13</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of present surgery</th>
<th>Laparotomy</th>
<th>1</th>
<th>3.3</th>
<th>2</th>
<th>6.7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cholecystectomy</td>
<td>12</td>
<td>40</td>
<td>8</td>
<td>26.7</td>
</tr>
<tr>
<td></td>
<td>Hernia</td>
<td>3</td>
<td>10</td>
<td>8</td>
<td>26.7</td>
</tr>
<tr>
<td></td>
<td>Gastric surgery</td>
<td>4</td>
<td>16.7</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>10</td>
<td>33.3</td>
<td>9</td>
<td>30</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100</td>
<td>30</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Duration of surgery</th>
<th>≤1:30</th>
<th>20</th>
<th>66.7</th>
<th>24</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;1:30</td>
<td>10</td>
<td>33.3</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100</td>
<td>30</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>
and about control group (56.7%) have previous surgery (16.7%) of them with Caesarian surgery. Regarding type of present surgery the majority of the case group (40%) have cholecystectomy surgery, and (26.7) of control group have share cholecystectomy and hernia surgery. Concerning duration of surgery (80%) in control group is more than 1:30 hours while the case group is (66.7%).

**Table (2) Differences Between the Lungs Volumes for the Case and Control Group Through Pre-Operative Periods n=60**

<table>
<thead>
<tr>
<th>Lungs volumes</th>
<th>Pairs</th>
<th>Mean</th>
<th>N</th>
<th>Std. Deviation</th>
<th>T-value</th>
<th>d.f</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV1</td>
<td>Case</td>
<td>3.3173</td>
<td>30</td>
<td>0.81448</td>
<td>0.311</td>
<td>58</td>
<td>0.757 NS</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>3.2493</td>
<td>30</td>
<td>0.87842</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FVC</td>
<td>Case</td>
<td>3.9960</td>
<td>30</td>
<td>1.02234</td>
<td>0.149</td>
<td>58</td>
<td>0.0882 NS</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>3.9577</td>
<td>30</td>
<td>0.97663</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV1%</td>
<td>Case</td>
<td>81.9163</td>
<td>30</td>
<td>4.12529</td>
<td>0.618</td>
<td>58</td>
<td>0.539 NS</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>80.9903</td>
<td>30</td>
<td>7.09822</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (2) shows that is non-significant difference between the levels of all the lungs’ volumes between the case and control group at the pre-test in pre-operative period.

**Table (3) Differences Between two Periods of Measurements of Lungs Volumes for Case and Control Group Through Post-Operative Periods n=60**

<table>
<thead>
<tr>
<th>Lungs volumes</th>
<th>Periods of measurement</th>
<th>Pairs</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>T-value</th>
<th>d.f</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV1</td>
<td>Post-test 1 (after 8 hour)</td>
<td>Case</td>
<td>2.0243</td>
<td>0.41163</td>
<td>2.840</td>
<td>58</td>
<td>0.006 HS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>1.6723</td>
<td>0.53972</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post-test 2 (after 24 hour)</td>
<td>Case</td>
<td>2.5447</td>
<td>0.41462</td>
<td>4.795</td>
<td>58</td>
<td>0.001 HS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>2.0137</td>
<td>0.44264</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FVC</td>
<td>Post-test 1 (after 8 hour)</td>
<td>Case</td>
<td>2.6630</td>
<td>0.47391</td>
<td>2.466</td>
<td>58</td>
<td>0.017 S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>2.2943</td>
<td>0.66790</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post-test 2 (after 24 hour)</td>
<td>Case</td>
<td>3.1487</td>
<td>0.49057</td>
<td>3.676</td>
<td>58</td>
<td>0.001 HS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>2.6823</td>
<td>0.49205</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV1%</td>
<td>Post-test 1 (after 8 hour)</td>
<td>Case</td>
<td>76.4360</td>
<td>7.71847</td>
<td>2.076</td>
<td>58</td>
<td>0.043 S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>71.6333</td>
<td>10.0527</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post-test 2 (after 24 hour)</td>
<td>Case</td>
<td>80.0577</td>
<td>6.32315</td>
<td>2.039</td>
<td>58</td>
<td>0.046 S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>76.1113</td>
<td>8.50237</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (3) shows there are a statically differences between studied groups according level of lung volume, P- value were less than 0.006) in two period of measurement post-operatively).
Table (4) Correlation between Lungs Volume and Socio-Demographic and Clinical Data of Case Group in Post-test 2 (after 24 hour) N= 30

<table>
<thead>
<tr>
<th>Rating and Intervals</th>
<th>lungs volumes</th>
<th>r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>FEV1</td>
<td>-0.895</td>
<td>0.001 HS</td>
</tr>
<tr>
<td></td>
<td>FVC</td>
<td>-0.619</td>
<td>0.002 HS</td>
</tr>
<tr>
<td></td>
<td>FEV%</td>
<td>-0.416</td>
<td>0.003 HS</td>
</tr>
<tr>
<td>Body mass index</td>
<td>FEV1</td>
<td>-0.722</td>
<td>0.041 S</td>
</tr>
<tr>
<td></td>
<td>FVC</td>
<td>-0.426</td>
<td>0.016 HS</td>
</tr>
<tr>
<td></td>
<td>FEV%</td>
<td>-0.399</td>
<td>0.001 HS</td>
</tr>
</tbody>
</table>

Table (4) shows there are a strong negative correlation between patient’s lungs volumes (FEV1, FVC,) and age (r=-0.895;0.001) (-0.619; 0.001) respectively, FEV1 and body mass index (r = 0.722; 0.041), as well as a significant correlation among other studied parameters.

Discussion

Regarding Table (1) there was a high percentage of patients at age groups (50-59) years old, and the majority of study samples were male. This could be agreeable with 11 as their study have the same range age group, and the gender was also mainly male. The present results show that most patients are primary school graduate, many people might live with number of social and economic barriers to stressful and conflict environment, this can prevent them for achieving higher educational levels, this result could be supported by 12 majority of their study are primary school graduate were (78.5%). It is show that there is non-significant difference between both groups in the baseline pulmonary functions parameters mentioned in the pre-operative period. This may be due to both groups were had resemble characteristic and functionally comparable to each other. The present result is similar to a study conducted by 13,14 are similar with the result of present study, and the pulmonary function test parameters did not differ between two groups before operation. After the application of the breathing exercise pursed lip methods through the present study, the results indicated that there is an improvement in the case group lungs volumes compared with those patients in the control group. The result shows that there is a significant difference between groups. Patients in the study group who demonstrate breathing exercise had a higher pulmonary function in all parameters than in control group at P- value less than 0.05 in all the post-operative periods. The applied method is an effective way to improve the post-operative patients’ lung function. The results of this study agree with the observations of 15, they concluded that the pre-operative deep breathing exercise improves the pulmonary functions, with significantly improved for lungs parameters such as FVC, FEV1 at P-value = 0.003, and P-value less than 0.001 respectively). The study shows that there is a relationship between the patient’s lung volume with their age and body mass index at p-value less than 0.05 after 24 hours postoperatively, while the other results indicate there is no relationship between patients lungs volumes and other demographic and clinical data at p-value more than 0.05. Pulmonary function may decrease and affected by age. The rate of respiratory and blood circulation are increases in childhood and become in a maximum level at age group between (20–30) years, then decline again in the older age. About high body mass index there are some reasons for decline the lung function. Firstly, the diaphragm position in the thoracic cavity is elevated obviously when individual increase weight. Secondly, it appears that fat accumulation on the chest wall will impede the movement of thoracic cage by a direct resistance or the abnormal function of intercostal muscle 16,17.

Conclusion

There were no differences between lung function in both case and control groups in the pretest. There is an improvement in the postoperative patients’ lungs function in study group after exposure to an educational breathing exercise program. The program show obvious difference between the lung function for both study and control groups during the post-test. Control group does not present any modification in their lung function post-
operatively. In addition, there is relation between study groups and demographical data in age and body mass index only.

**Conclusion**

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the University of Kufa. Faculty of Nursing, Iraq and all experiments were carried out in accordance with approved guidelines.

**References**


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Glomerular Diseases: Systemic Lupus Erythematosus is Most Common Finding: A Cross-Sectional Study

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Abstract

The objective of this study was to assess the connection amongst’s immunological and histopathological findings of renal biopsy acquired from patients with glomerular diseases. This cross sectional study was done in Al-Kafil private hospital and included 110 patients who were diagnosed by nephrologist as having glomerulonephritis as per clinical introduction, physical examination and laboratory findings of biopsy. The age scope of patients enlisted in the present investigation was between 2.8 to 72 years. The study included 67 male patients (60.9%) and 43 female patients (39.1%). The examination began at January 2016 and reached out through January 2017. At the point when clinical introduction and the entire workup of examination were at long last joined, with exceptional accentuation on histopathology and immunofluorescent tiny examination, the accompanying classification was gotten. Cases with glomerular association optional to foundational lupus erythematosis represented 27 out of 110 (24.5%). Consequently lupus nephritis is the most incessant reason for glomerulonephritis observed in the present examination. As indicated by the International Society of Nephrology (ISN)/Renal Pathology Society (RPS) grouping of lupus nephritis, the most incessant compose was ISN/RPS class IV (A/C) represented 14 case (12.7%), trailed by ISN/RPS class III (A/C) (8 case, 7.3%)

Key words: Glomerular disorders, SLE, immunoflourescent, Iraq

Introduction

The kidney in spite of the fact that being moderately little size in examination with add up to body weight plays out a great deal of capacities that keep human body at ideal physiological conditions. These capacities incorporate water and electrolyte adjust, corrosive base direction, endocrine capacities and discharge of waste items, for example, urea and creatinine. Infection influencing the kidney may include the veins, glomeruli and tubulointerstitial compartments. Glomerular sicknesses represent a huge extent of dreariness and mortality in the populace. Glomerular sicknesses are regularly because of some type of immunological affront. Immunological bases for glomerular damage can be expected do humeral or cell invulnerable reaction. Humeral resistant reaction includes a counter acting agent that is independent from anyone else poisonous to certain segment of the glomerulus, for example, Good Pasteur disorder in which the immunizer is coordinated against glomerular cellar layer. Then again humeral reaction might be because of affidavit of insusceptible edifices that are either framed in situ or effectively flowing in blood, for example, glomerular damage found in relationship with foundational lupus erythematosus (SLE). Likewise, humeral insusceptible reaction might be because of enactment of elective supplement pathway. The established introduction of glomerular sickness is as proteinuria and/or hematuria. The time of beginning is exceptionally factor and is amazingly wide so glomerular ailment might be found in youngsters, immature, youthful grown-up and elderly people. The particular order of the sort of glomerular damage is basic for both treatment system portion and guess of
the sickness to be cleared up. For distinct conclusion of glomerular ailment, renal biopsy ought to be performed. Three principle steps take after renal biopsy got for suspected glomerular ailments; these are normal and extraordinary histogical stains, electron minute examination and immunofluorescent tiny examination. The innovation of tissue immunofluorescent strategy, by Coons and Kaplan in 1950 and its application on renal biopsy by Mellors in 1955, constrained the requirement for electron microscopy in symptomatic renal biopsy. Immunofluorescence allows the recognizable proof of the guilty party immunoglobulin (IgG, M and An) and furthermore the included supplement segment (C3 and C1q) and furthermore allow the distinguishing proof of safe testimony whether membranous or mesangial. In Iraq there is extremely set number of concentrates that managed this subject and the greater part of studies took a set number of cases and was constrained to a solitary or various glomerular issue. The vast majority of these examinations featured the histogical adjustment as well as clinical angles. The absence of an Iraqi immunologic investigation that arrangements with extensive example and different sorts of glomerular infections defended the conduction of the streams think about.

The Aim of this study was to assess the connection amongst’s immunological and histopathological discoveries of renal biopsy acquired from patients with glomerular infection.

**Patients and Method**

This cross sectional examination was done in Al-Kafil private doctor’s facility and included 110 patients who were analyzed by nephrologist as having glomerulonephritis as per clinical introduction, physical examination and discoveries of research center examinations. The age scope of patients enlisted in the present investigation was between 2.8 to 72 years. The example included 67 male patients (60.9%) and 43 female patients (39.1%). The examination began at January 2016 and reached out through January 2017. The procedure of patients’ determination begun at the nephrology meeting room. Any patient having the accompanying highlights was incorporated into the present investigation:

- Patients with regular picture of nephritic disorder
  - Massive proteinuria, summed up edema, hypoalbuminemia, hyper-lipidemia and lipiduria.
  - Patients with regular highlights of nephritic disorder
    - Hematuria, hypertension, azotemia and oliguria
  - Patients with highlights that are not suggestive of glomerular association; in any case, urinalysis of them indicated critical proteinuria as well as glomerular hematuria
  - Patients with intense or constant disappointment with extra clinical highlights suggestive of glomerular malady

Any patient with highlights that are not suggestive of glomerular sickness and have no proof of huge proteinuria and/or glomerular heamturia was barred from the investigation. Routine histological appraisal, immunoflourescent ponder were the fundamental examination to which all patients were subjected, adjacent to hematological, serological, biochemical examinations and urinalysis.

**Results**

Demographic characteristics of the study sample

The present study included 110 patients with glomerulonephritis, 67 male patients (60.9%) and 43 female patients (39.1%) with a male to female ratio of 1.56:1. Mean age of patients enrolled in the current study was $31.28 \pm 13.83$ years and it ranged from 2.8 to 72 years, as shown in table 4.1. According to 10 years age intervals patients were distributed as following: 2 patients less than 10 (1.8%), 21 patients from 11-20 (19.1%), 39 patients from 21-30 (35.5%), 23 patients from 31-40 (20.9%), 13 patients from 41-50 (11.8%), 6 patients from 51-60 (5.5%), 5 patients from 61-70 (4.5%) and a single patient older than 71 (0.9%), as shown in figure 1.
Mean duration of disease is 1.88 ± 1.66 years and it ranged from 2 weeks to 7 years. Patients with disease duration of less than one year accounted for 34 (30.9%), patients with 1 to less than 2 years accounted for 19 (17.3%), patients with disease duration of 2 to less than 3 years accounted for 17 (15.5%), patients with disease duration of 3 to less than 4 years accounted for 22 (20%), patients with disease duration of 4 to less than 5 years accounted for 9 (8.2%), patients with disease duration of 5 to less than 6 years accounted for 7 (6.4%), patients with disease duration of 6 to 7 years accounted for 2 (1.8%).

**Lupus nephritis**

Cases with glomerular involvement secondary to systemic lupus erythematosus accounted for 27 out of 110 (24.5%). Hence lupus nephritis is the most frequent cause of glomerulonephritis observed in the present study. According to the International Society of Nephrology (ISN)/Renal Pathology Society (RPS) classification of lupus nephritis, the most frequent type was ISN/RPS class IV (A/C) accounted for 14 case (12.7%), followed by ISN/RPS class III (A/C) (8 case, 7.3%) and lastly by ISN/RPS class III (A) (5 cases, 4.5%). Immunofluorescent study is shown in figure 2 and 3 in which there was mild diffuse segmental granular mesangial deposition of IgG and mild diffuse segmental granular mesangial deposition of IgM.

![Figure 2: Section of renal biopsy from patient with lupus nephritis stained with IgG immunofluorecence showing mild diffuse segmental granular mesangial deposition of IgG (red arrow) (10 X).](image-url)
Discussion

In this investigation Cases with glomerular association optional to foundational lupus erythematosis were the most regular and represented 27 out of 110 (24.5%); the most incessant write was ISN/RPS class IV (A/C) represented 14 case (12.7%), trailed by ISN/RPS class III (A/C) (8 case, 7.3%) and ultimately by ISN/RPS class III (A) (5 cases, 4.5%). In one Iraqi investigation, it was demonstrated that 25 (45.5%) cases were because of lupus nephritis 14 and in another Iraqi examination just two cases (3.4%) satisfied the clinical, serological and histopathological criteria of lupus nephritis 1. We concur with 14 that lupus nephritis is the most incessant reason for glomerulonephritis in Iraq and can’t help contradicting 1 who depicted a predetermined number for lupus nephritis in his investigation. In another examination in India, 3 assessed the clinicopathologic parts of crescentic glomerulonephritis and found that 14.7% of cases were because of SLE. Lupus renal malady gives off an impression of being more common in certain ethnic gatherings 10, and this may clarify the high rate of lupus nephritis in Iraqi patients. A similar investigation of SLE in three ethnic gatherings announced that renal malady, which is characterized by American College of Rheumatology (ACR) criteria as persevering every day proteinuria of >500 mg within the sight of cell throws or biopsy proof of lupus nephritis, happened in 45% of African Americans, 42% of Chinese, and 30% of Caucasian patients, individually 10. Another multi-ethnic US companion of SLE patients revealed that renal malady happened in 51% of Africans and 43% of Hispanics however in just 14% of Caucasians 2. In a planned investigation of 216 Chinese patients with new beginning SLE, 31% patients had dynamic renal sickness as the underlying introduction 8. The general combined frequency of renal sickness was 60% at 5 years post-SLE finding. These investigations delineated that lupus renal association is more typical in Africans, Hispanics, and Chinese than in Caucasians 9.
Conclusion

At the point when clinical introduction and the entire workup of examination were at long last joined, with exceptional accentuation on histopathology and immunofluorescent tiny examination, the accompanying classification was gotten. Cases with glomerular association optional to foundational lupus erythematosis represented 27 out of 110 (24.5%). Consequently lupus nephritis is the most incessant reason for glomerulonephritis observed in the present examination. As indicated by the International Society of Nephrology (ISN)/Renal Pathology Society (RPS) grouping of lupus nephritis, the most incessant compose was ISN/RPS class IV (A/C) represented 14 case (12.7%), trailed by ISN/RPS class III (A/C) (8 case, 7.3%) and in conclusion by ISN/RPS class III (A) (5 cases, 4.5%). In Iraq, SLE is the fundamental driver of glomerular damage.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Al-Hindiyah primary health care centre / Karbula province / Iraq and all experiments were carried out in accordance with approved guidelines.

References

Synthesis of Diimidazole Has Pyrazole, Isoxazole and Pyrimidine Derivatives and Evaluation as Antibacterial

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Abstract

New diimidazole contains pyrazole , isoxazole and thiopyrimidine were synthesized by using simple methods. All compounds were synthesized by using hippuric acid (1) as stating material which was obtained from benzoil and glycine with. pyrazole derivatives (6a – b ) was obtained from reaction (5a – b) with hydrazine hydrate while reaction of (5a – b )with hydroxyl amine isoxazole derivatives (7a – b) were obtained,finally reaction of thiourea was reacted with 5a – b thiopyrimidine derivatives were get (8a –b) . FTIR and 1HNMR spectra were used to characterized derivatives several bacterial species like Pseudomonas aeruginosa , Staphylococcus aureus, and Acinetobacter baumanii were used to tested antibacterial activity

Keywords: pyrazole, isoxazole, imidazole, thiopyrimidine

Introduction

Heterocyclics were found good biological molecules like imidazole 1 molecules with five and six membered rings mostly are high activity 2 . All natural products with heterocyclics are important in biologically active system 3 and medicinal to approved drugs , imidazole derivatives in heterocyclic chemistry considered most important compounds and drugs 4 such as Antioxidant activity 5 anti-microbial (6-7), antiangiogenic 8 , antitumor 9 , analgesic 10 , pyrazoles and isoxoles play important role to synthesis biologically active drugs like Antifungal11 , antimicrobial12 , antitumor13 , analgesics14 , Anthelminthic15 , Ant pathogenic16 , anti-inflammatory17

Method

Synthesis of [(phenyl carbonyl)amino]acetic acid(1)

Glycine (0.1mol ) in 15ml of 1N NaOH was cooled to 5 and then benzoyl chloride (0.1 mole ) was added to cold drop wise . The reaction mixture was stirring for one hour. The aqueous layer was acidified with 2N HCl and the product was collected and recrystallized from ethanol and product was collected as white powder

Synthesis of (Z)- 2-phenyl -4-benzylidene oxazol-5(4H)-ones (2a - d)

To a mixture of compound (1) ( 0.01 mole ) , acetic anhydride (20 ml) and acetic acid (5 ml), (0.01 mol) of aromatic aldehyde was added. And the mixture was refluxed for 4hr and the temperature was reached to 80°C. The reaction mixture was allowed to cool. , then poured into crushed ice and it was allowed stirred for 30 min. the product was recrystallized from ethanol.

Synthesis of 3-(2-amino)-5-arylidine-2-phenyl-3,5-dihydro-4H-imidazol-4-one (3a – d)

To a mixture of (2a – d ) derivatives (0.01 mole) in (20ml) triethyl amine , (0.01 mole) of hydrazine was added. And then it was refluxed 2 h. after that the mixture was cooled to RT. And the desired compound was obtained after recrystallizing from ethanol.

Synthesis of 1-[(4Z)-4-(arylidene )-5-oxo-2-phenyl-4,5-dihydro-1H-imidazol-1-yl] thiourea (4a – d)

Potassium thiocyanate was added to a mixture of compounds [3a - d] (0.01 mole) in ethanol (27 ml) and then stirred for 30 min. the mixture was poured in to could water and then filtered, the solid (desired compound) was recrystallized from ethanol.

Synthesis of (5Z)-5-(4-chlorobenzylidene)-3-(4-oxo-2-thioxoimidazolidin-1-yl)-2-phenyl-3,5-dihydro-4H-imidazol-4-one (5a – d)

To solution of (4a – d) (0.01 mole), in 30 ml of dry benzene chloro acetyl chloride was added and the mixture was heated in water bath at (60 0C) for 3 hrs. with stirring after that the solvent was evaporated, and
the product desired compound was recrystallized from appropriate solvent.

Synthesis of 1-[3-(Aryl)-5-phenyl-3,3a-dihydroimidazo[4,5-c]pyrazol-6(2H)-yl]-2-thioxoimidazolidin-4-one (6a – b)

To solution of (5a – b) (0.01 mole), in 20 ml of acetic acid hydrazine hydrate was added and the mixture was heated (refluxed) for 5 hrs. with stirring after that mixture poured to water (200ml), and the product desired compound was recrystallized from appropriate solvent.

Synthesis of 1-[3-(Aryl)-5-phenyl-3,3a-dihydro-6H-imidazo[4,5-c]isoxazol-6-yl]-2-thioxoimidazolidin-4-one (7a – b)

To solution of (5a – b) (0.01 mole), in 20 ml of acetic acid hydroxyl amine hydrochloride was added and the mixture was heated (refluxed) for 5 hrs. with stirring after that mixture poured to water (200ml), and the product desired compound was recrystallized from appropriate solvent.

Results and Discussion

Scheme (1) shown all compounds were synthesized

\[
\text{Ar} = \text{p-NO}_2, \text{p-Cl}, \text{p-Br}, \text{p-NMe}_2
\]
Synthesis of target (1) was done by the reaction of amino acid with benzoyl chloride through nucleophilic mechanism. (93%). m.p (186-188), color (White). The FT-IR spectrum of compound (1), shows stretching vibration of (OH) of carboxylic acid at (2610-3390) cm⁻¹ and appearance of new band at (3350) cm⁻¹ for NH.

Compound (1) was treated with aryl lead to formation of compounds (2a - d)

2a : yield (79%), FT-IR cm⁻¹ C=N(1646), C=O(1729), (C=C)ar (1601,1551), (C-H)ar (3083), (NO₂)(1534-13), 1H-NMR(ppm), s,6.92 for(C=CH), (7.25-7.76) (m, aromatic protons).

2b : yield (82%), FT-IR cm⁻¹ C=N(1646), C=O(1729), (C=C)ar (1601,1551), (C-H)ar (3083), (NO₂)(1534-13), 1H-NMR(ppm), s, (6.88) for(C=CH), (7.25-7.76) (m, aromatic protons).

2c : yield (75%), FT-IR cm⁻¹ C=N(1646), C=O(1729), (C=C)ar (1601,1551), (C-H)ar (3083), (NO₂)(1534-13), 1H-NMR(ppm), s, (6.76) for(C=CH), (7.25-7.76) (m, aromatic protons).

2d : yield (80%), FT-IR cm⁻¹ C=N(1646), C=O(1729), (C=C)ar (1601,1551), (C-H)ar (3083), (NO₂)(1534-13), 1H-NMR(ppm), s, (6.51) for(NMe₂), s, (5.67) for(NH₂), s, (6.48) for(C=CH), (7.25-7.76) (m, aromatic protons).

Treatment of (2a – d) with hydrazine gives (3a – d)

3a : yield (78%), FT-IR cm⁻¹ NH₂ (3345,3213), C=N(1656), C=O(1701), (C=C)ar (1601,1553), (C-H)ar (3079), (NO₂)(1545-1351), 1H-NMR(ppm), s, (5.66) for(NH₂), s, (5.68) for(C=CH), (7.11-7.69) (m, aromatic protons).

3b : yield (69%), FT-IR cm⁻¹ NH₂ (3401,3234), C=N(1661), C=O(1709), (C=C)ar (1600,1500), (C-H)ar (3060), (C=Cl), (1109), 1H-NMR(ppm), s, (5.23) for(NH₂), s, (5.31) for(C=CH), (7.51-7.77) (m, aromatic protons).

3c : yield (73%), FT-IR cm⁻¹ NH₂ (3389,3143), C=N(1666), C=O(1712), (C=C)ar (1599,1501), (C-H)ar (3069), (C-Br) 977, 1H-NMR(ppm), s, (5.46) for(NH₂), s, (6.76) for(C=CH), (7.55-7.92) (m, aromatic protons).

3d : yield (61%), FT-IR cm⁻¹ NH₂ (3345,3207), C=N(1643), C=O(1718), (C=C)ar (1607,1500), (C-H)ar (3070), (C-H) alph (2879-2980), 1H-NMR(ppm), s, (3.61) for(NMe₂), s, (5.66) for(NH₂), s, (6.48) for(C=CH), (7.11-7.69) (m, aromatic protons).

Reaction of compounds (3a – d) with KSCN in ethanol, compounds (4a – d) was formed

4a : yield (71%), FT-IR cm⁻¹ NH₂ (3311,3225), NH (3112), C=N(1646), C=O(1731), (C=C)ar (1601,1551), (C-H)ar (3083), (NO₂)(1534-13), 1H-NMR(ppm), s, (9.21) for(NH), s, (8.47) for(NH₂), s, (6.43) for(C=CH), (7.19-7.68) (m, aromatic protons).

4b : yield (56%), FT-IR cm⁻¹ NH₂ (3432,3267), NH (3152), C=N(1657), C=O(1723), (C=C)ar (1603,1500), (C-H)ar (3035), (C-Cl), (1097), 1H-NMR(ppm), s, (9.71) for(NH), s, (8.62) for(NH₂), s, (6.53) for(C=CH), (7.33-7.78), (m, aromatic protons).

4c : yield (60%), FT-IR cm⁻¹ NH₂ (3415,3270), NH (3165), C=N(1643), C=O(1715), (C=C)ar (1604,1509), (C-H)ar (3065), (C-Br) 975, 1H-NMR(ppm), s, (9.22) for(NH), s, (8.41) for(NH₂), s, (6.69) for(C=CH), (6.79-7.94) (m, aromatic protons).

4d : yield (58%), FT-IR cm⁻¹ NH₂ (3411,3272), NH (3149), C=N(1632), C=O(1708), (C=C)ar (1603,1508), (C-H)ar (3066), (C-H) alph (2884-2987), 1H-NMR(ppm), s, (3.54) for(NMe₂), s, (9.49) for(NH), s, (8.61) for(NH₂), s, (6.59) for(C=CH), (6.76-7.85) (m, aromatic protons).

Cyclization of compounds (4a – d) with chloroacetyl chloride gives compounds (5a – d)

5a : yield (75%), FT-IR cm⁻¹ NH₂ (3415,3270), NH (3110), C=N(1638), C=O(1721), C=O thio imidazole (1751), 1H-NMR(ppm), s, (4.21) for(CH₂), thio imidazole, s, (9.23) for(NH), s, (6.62) for(C=CH), (7.71-7.90) (m, aromatic protons).

5b : yield (68%), FT-IR cm⁻¹ NH₂ (3176), C=N(1650), C=O(1702), C=O thio imidazole (1757), C=O thio imidazole (1757), (C=C)ar (1612,1509), (C-H)ar (3067), (C-Cl), (1019), 1H-NMR(ppm), s, (4.25) for(CH₂), thio imidazole, s, (9.11) for(NH), s, (6.59) for(C=CH), (7.18-7.73) (m, aromatic protons).

5c : yield (60%), FT-IR cm⁻¹ NH₂ (3134), C=N(1657), C=O(1728), C=O thio imidazole (1749), (C=C)ar (1600,1501), (C-H)ar (3070), (C-Br) 912, 1H-NMR(ppm), s, (4.42) for(CH₂), thio imidazole, s, (9.68) for(NH), s, (6.68) for(C=CH), (7.26-7.53) (m, aromatic protons).
5d : yield (58%), FT-IR cm\(^{-1}\) NH (3190), C=N(1648), C=O (1716), C=O thio imidazole (1746) (C=C)ar (1615,1529), (C-H)ar (3079), (C-H) aliph. (2879-2956). 1H-NMR (ppm), s, (3.29) for (NMe\(_2\)), s, (4.22) for (CH\(_2\), thio imidazole), s, (9.39) for (NH), s, (6.14) for (C=CH), (7.17-7.65) (m, aromatic protons).

Compounds (6a – b), (7a – b) and (8a – b) were obtained by cyclizing compounds (5a – b) with hydrazine, hydroxyl amine and thiourea respectively.

6a : yield (65%), FT-IR cm\(^{-1}\), NH (3213), NH of pyrazole, (3171), C=N (1645), C=O thio imidazole (1735), (C=C)ar (1611,1500), (C-H)ar (3060), (NO\(_2\)), (1555-1353). 1H-NMR (ppm), s, (4.29) for (CH\(_2\), thio imidazole), s, (9.22) for (NH), s, (8.27) for (NH, pyrazole), (7.53-7.98) (m, aromatic protons).

6b : yield (61%), FT-IR cm\(^{-1}\), NH (3269), NH of pyrazole, (3199) C=N(1661), C=O thio imidazole (1739) (C=C)ar (1600,1501), (C-H)ar (3071), (C-Cl), (1021). 1H-NMR (ppm), s, (4.19) for (CH\(_2\), thio imidazole), s, (9.82) for (NH), s, (8.79) for (NH, pyrazole), (6.89-7.24) (m, aromatic protons).

7a : yield (71%), FT-IR cm\(^{-1}\), NH (3245), C=N (1651), C=O thio imidazole (1743), (C=C)ar (1605,1516), (C-H)ar (3079), (NO\(_2\)), (1532-1350). 1H-NMR (ppm), s, (4.02) for (CH\(_2\), thio imidazole), s, (9.46) for (NH), (6.92-7.48) (m, aromatic protons).

7b : yield (77%), FT-IR cm\(^{-1}\), NH (3211), C=N(1670), C=O thio imidazole (1728) (C=C)ar (1609,1511), (C-H)ar (3079), (C-Cl), (1011), 1H-NMR (ppm), s, (4.10) for (CH\(_2\), thio imidazole), s, (9.32) for (NH), (6.89-7.24) (m, aromatic protons).

8a : yield (57%), FT-IR cm\(^{-1}\), NH (3311), NH of pyrimidine, (3165) C=N (1649), C=O thio imidazole (1741), (C=C)ar (1601,1511), (C-H)ar (3092), (NO\(_2\)), (1539-1355). 1H-NMR (ppm), s, (4.20) for (CH\(_2\), thio imidazole), s, (9.71) for (NH), s, (8.16) for (NH, pyrimidine), (6.72-7.30) (m, aromatic protons).

8b : yield (54%), FT-IR cm\(^{-1}\), NH (3302), NH of pyrimidine, (3190) C=N(1644), C=O thio imidazole (1733) (C=C)ar (1600,1500), (C-H)ar (3085), (C-Cl), (1077). 1H-NMR (ppm), s, (4.51) for (CH\(_2\), thio imidazole), s, (9.76) for (NH), s, (8.36) for (NH, pyrimidine), (6.99-7.59) (m, aromatic protons).

Table 1. Antibacterial Activity of derivatives (4a – d, 5a – d, 6a – b, 7a – b and 8a – b).

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Staph. aureus</th>
<th>E. coli</th>
<th>Proteus mirabilis</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>++++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>4b</td>
<td>++</td>
<td>++++</td>
<td>++</td>
</tr>
<tr>
<td>4c</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>4d</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>5a</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>5b</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>5c</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>5d</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>6a</td>
<td>+++</td>
<td>++++</td>
<td>+++</td>
</tr>
<tr>
<td>6b</td>
<td>++</td>
<td>++++</td>
<td>++</td>
</tr>
<tr>
<td>7a</td>
<td>+</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>7b</td>
<td>++</td>
<td>++++</td>
<td>++</td>
</tr>
<tr>
<td>8a</td>
<td>+++</td>
<td>++++</td>
<td>+++</td>
</tr>
<tr>
<td>8b</td>
<td>++++</td>
<td>++++</td>
<td>+++</td>
</tr>
</tbody>
</table>
Antibacterial activity of derivatives

It might be observed that derivatives which, when tested, were active but 8a, 8b, 4b and 6a have high activity toward all types of tested bacterial while compound 5a and 5d have weak activity toward all types of tested bacteria. Compound 8a showed high inhibition with E. coli and Proteus mirabilis.

Conclusion

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Faculty of Dentistry, University of Babylon, Hillah city, Iraq, and all experiments were carried out in accordance with approved guidelines.

References

1. AJK Atia Synthesis and antibacterial activities of new metronidazole and imidazole derivatives, Molecules 2009;14(7):2431-2446
Novel approach and Cloud Point Extraction Method for Determination of Acetazolamide Drug

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1Chemistry Department, College of Science, Babylon University, Iraq

Abstract

Acetazolamide was hydrolyzed to primary aromatic amine by using (0.4) M NaOH under reflux. The product was evaluated by two ways. The first way, Diazotization coupling reaction (approach) as simple, sensitive, rapid and selective Spectrophotometric method, using 8-hydroxyquinoline as Chromogenic reagent to give Azo dye (red) in basic medium. The second way involves applying Cloud point extraction, using Triton-x114 as surfactant. The Azo dye was diagnosed by FT-IR, 1HNMR and UV-Visible technique. The analytical data for Approach and Cloud point extraction method, involve concentration rang (5-150), (0.5-6) µg.mL-1, molar absorptivity (2.3×103), (1.3×104) L.mol^-1.cm^-1, Sandall’s sensitivity (0.096) µg.cm^-2 (0.017) µg.cm^-2 and detection limits (0.952) µg.mL^-1 and (0.043) µg.mL^-1 respectively. In addition the measurement enrichment factor (100) and preconcentration factor (6.30), The proposed methods don’t affect by the existence of excipients so the methods were applied successfully in determining Acetazolamide in pharmaceutical preparations.

Keywords: Hydrolysis Acetazolamide, spectrophotometric determination, Cloud point extraction, Diazotization coupling reaction, 8-Hydroxyquinoline.

Introduction

Acetazolamide a carbonic anhydrase inhibitor, which is used primarily to reduce intraocular pressure by decreasing aqueous humor formation, therapeutically for treatment of glaucoma, epilepsy and as a diuretic and has been used clinically since 1954 1-4. Acetazolamide in either medicinal forms or biological fluids were estimated in several techniques and methods have been declared in the literature, including HPLC for the quantification of acetazolamide in human and rat plasma 5-7, LC/MS and GC/MS 8-10, LC-UV 11-13, and spectrophotometry for determination acetazolamide and other sulfonamide drugs 14-19. Applications of cloud point extraction techniques for estimation of some elements and drugs 20,21. The present paper involves a novel determination of acetazolamide in pure and pharmaceutical formulations by Diazotization coupling reaction (approach) and Cloud point extraction spectrophotometric method, depending on basic hydrolysis of acetazolamide to primary aromatic amine, and coupling the product with 8-Hydroxyquinoline as a chromogenic reagent to give Azo-dye in alkaline medium.

Experimental

Instruments

The scanning of all spectrums and measurements of the absorbance at selected wavelengths achieved by T80 UV-Visible Spectrometer PG Instrumental Ltd, UK, with quartz cell matched 1 cm, Infrared spectra were registered using FT-IR, Shimadzu, Japan, 1HNMR spectrum was registered using NMR Burker DPX 400 spectrophotometer operating at 300 MHz. the chemical shift δ is quoted in ppm relative to DMSO-d6, while the pH was adjusted using 340i pH-meter WTW, Germany, and Heating-Cooling Water Bath – Haak Fe, Sartorius

Chemicals and reagents

Highest purity of Acetazolamide (C4H6N4O3S2) was gained from state company for drug Industries and Medical Appliance-(SDI) Samarra-Iraq, 8-hydroxyquinoline (C8H7NO),Sodium nitrite (NaNO2),Absoluteethanol(C2H5OH), Sodium hydroxide (NaOH), Sodium carbonate (Na2CO3) were gained from the BDH Company with Purity 99.00%. Hydrochloric acid (HCl) was gained from a BDH Company with
concentration 37.00%. Triton X-114 from Arcos organics, New Jersey, USA with purity 100%. Pharmaceutical preparations that were used in this study were Cidamex and Diamox 250mg/acetazolamide from CID Egypt and France respectively.

Hydrolysis of Acetazolamide

0.1 g of Acetazolamide was hydrolyzed in alkaline medium by using 0.4 M NaOH under reflux for 2 hours. During the reaction, the secondary amide is converted to the primary amine. The synthetic path of the product is shown in Scheme I. The hydrolysis product was cooled and diluted to suitable volume using distilled water to obtain a stock solution (2000 µg mL⁻¹). More dilute solution was prepared daily by neutralize of stock solution with dilute hydrochloric acid and dilute to final suitable volume using distilled water. The tablet sample (Cidamex and Diamox 250mg/acetazolamide) were prepared in the same manner.

2 mol. from the hydrolysis product was converted into dizonium salt ion by using 0.3 mL HCl 1 M and 0.2 mL NaNO₂ 1%. The dizonium salt ion was coupled with 1 mol. 8-hydroxyquinoline in alkaline medium to give azo-dye. The synthetic path of the azo-dye is shown in Scheme II. The azo-dye was precipitate, purified and diagnosed by FT-IR, ¹H-NMR and UV-Visible technique as shown in Fig.2, 3 and 4.

Identification of the prepared drug and Azo Dye

Identification of the Hydrolysis product and Azo Dye. The hydrolysis product was prepared as explained in paragraph 2.3. The qualities experimental were involved tests for amine group by using Nitrous acid test and Azo-dye test. The hydrolysis product (5-amino-1,3,4-thiadiazole-2-sulfonamide) give yellow solution result from a reaction between a primary aromatic amine with concentrated HCl and NaNO₂ to formation diazonium salt ion, while the acetazolamide before hydrolysis not reaction. The two compounds were tested also by azo-dye test. The hydrolysis product after formation diazonium salt ion was coupled with 8-Hydroxyquinoline in alkaline medium to give azo-dye, while the acetazolamide before hydrolysis not reaction. This azo-dye was prepared by taking a stoichiometric amounts was precipitate, purified and diagnosed by FT-IR, ¹H-NMR and UV-Visible technique.

IR spectrum of azo-dye showed in Fig.1. one peak at 3417 cm⁻¹ assigned for stretching (O-H) of phenolic group, peak at 1426 cm⁻¹ assigned of stretching (N=N), and other peak 1575, 1145 and 879 cm⁻¹ assigned for stretching (C=N) thiadiazole, (S-C) and (C-S-C) groups respectively. This mean the formation of the aromatic primary amine group by hydrolysis of Acetazolamide and converted the amine in to diazonium salt in the presence of HNO₂ and coupling the last with 8-Hydroxyquinoline in alkaline medium. The ¹H-NMR spectrum (DMSO-d₆, 400 MHz) of azo-dye complex showed chemical shifts at δ 1.66 (1H, -SO₂NH₂), Singlet broad band at δ 3.5 refer to water in the solvent, DMSO, where its band δ 2.5, δ 5.5 (1H, aromatic -C-OH), δ 7.1(2H,C-H₅).
quinoline), δ 7.4 and 7.5 (3H,2H, C-H$_{22}$ quinoline), δ 8.3 and 8.85 (2H,1H, C-H$_{21}$ quinoline) 26-28 as shown in Fig.1. The qualitative and quantitative study of drug after hydrolysis, also done by UV-Visible technique. The Azo-dye complex (color product) scanned at (700-400 nm), and give a maximum absorption $\lambda_{\text{max}}$ at (502 nm) versus blank solution. while the scan of the blank solution versus water doesn’t give any absorption at $\lambda_{\text{max}}$ for colored product as shown in Fig.3. This property was adopted in the estimation of trace amounts from Acetazolamide in pure and pharmaceutical preparations.

Figure 1. Identification of Azo Dye by FT-IR, $^1$H-NMR and UV-Vis A: Azo-dye in Batch method, B: Azo-dye in Cloud point extraction method
Optimization of Experimental Conditions

Selection Optimal Experimental Conditions of Approach method

The effect of various experimental conditions as concentration, volumes from 8-Hydroxyquinoline reagent, volumes of HCl (1M), volumes of (1%) Sodium Nitrate, different types of bases, various volumes of Sodium Hydroxide (0.4 M) and temperatures of formation of (40 µg.ml⁻¹) azo dye were studied. The starting point involves using (0.5mL) from (1M) Hydrochloric acid with (0.5 mL) from (1%) Sodium nitrate, stand for (5 min), then (0.5mL) from (0.05M) 8-Hydroxyquinoline, and (0.5mL) from (0.4 M) Sodium hydroxide were added in (10 mL) volumetric flask. The absorbance of the solutions was measured at (λmax=502nm) against blank solutions after (5 min) since the beginning of the coupling reaction. The effect of various experimental conditions shown in Fig.2.

Selection Optimal Experimental Conditions of CPE method

The effect various concentrations, volumes of 8-Hydroxyquinoline reagent, volumes of HCl (1M), optimum pH value, volume of Triton X-114 5%, also effect of temperature and incubation time on formation of surfactant of (5 µg.ml⁻¹) azo dye were studied. The starting point involves (0.3 mL) of Hydrochloric acid (1M) fellow (0.2 mL) of Sodium nitrate (1%), stand for (5 min ), then (0.3 mL) from 8-Hydroxyquinoline (0.005M), at (pH = 7) Sodium carbonate with (0.3 mL) Triton X-114 5% were added in (10 mL) volumetric flask. The mixture was heated (10 min) at 55°C in water bath. The mixture was separated by centrifuge Ramp=3500 at (10 min). The surfactant rich phase was diluted with (0.3 mL) absolute ethanol and the absorbance of the solution was measured at (λmax=502nm) against the blank solution The effect of various experimental conditions shown in Fig.2.

Figure 2 The effect of various experimental conditions on formation of Azo dye
Calibration curve

After fixing all the optimum conditions for the reaction of acetazolamide after hydrolysis with 8-Hydroxyquinoline of approach and cloud point extraction method the calibration curves were constructed as shown in Fig.3. The all analytical values were calculated with accuracy and precision result are summarized in Table.1. The results shown each method has a good accuracy and precision.

Table.1 Summary of analytical value of calibration curves with accuracy and precision resulting of approach and CPE method with

<table>
<thead>
<tr>
<th>N</th>
<th>Parameter</th>
<th>Value</th>
<th>Approach method</th>
<th>CPE method</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Regression equation</td>
<td>Y= 0.0103x+0.0099</td>
<td></td>
<td>Y= 0.0593x+0.0024</td>
</tr>
<tr>
<td>2</td>
<td>Slope</td>
<td>0.0103</td>
<td></td>
<td>0.0593</td>
</tr>
<tr>
<td>3</td>
<td>Intercept (a)</td>
<td>0.0099</td>
<td></td>
<td>0.0024</td>
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<tr>
<td>4</td>
<td>Correlation coefficient</td>
<td>0.9981</td>
<td></td>
<td>0.9992</td>
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<tr>
<td>5</td>
<td>Linear Range (µg.mL⁻¹)</td>
<td>5-150</td>
<td></td>
<td>5.6</td>
</tr>
<tr>
<td>6</td>
<td>Molar absorptivity (E) (L.mol⁻¹.cm⁻¹)</td>
<td>2.3×10⁻³</td>
<td></td>
<td>1.3×10⁻⁴</td>
</tr>
<tr>
<td>7</td>
<td>Sandall's sensitivity (S) (µg.cm⁻²)</td>
<td>0.096</td>
<td></td>
<td>0.017</td>
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<tr>
<td>8</td>
<td>Limit of Detection LOD (µg.mL⁻¹)</td>
<td>0.952</td>
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<td>0.043</td>
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<tr>
<td>9</td>
<td>Limit of Quantitation LOQ (µg.mL⁻¹)</td>
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<tr>
<td>10</td>
<td>Preconcentration factor</td>
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<td>100</td>
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<tr>
<td>11</td>
<td>Enrichment factor</td>
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<td>6.3</td>
</tr>
</tbody>
</table>

Stoichiometry of Reaction and mechanism

The methods of Mole ratio and Continuous variation, Jobs method were used to detect the stoichiometry of azo-dye formation from reaction 8-Hydroxyquinoline reagent with acetazolamide drug. The results obtained in Fig.4 shown that 2:1 acetazolamide to 8-hydroxyquinoline was formed at 502 nm. And The average conditional stability constant of the colored products in water at optimum conditions was $6 \times 10^{12}$ L² mol⁻². The azo dye has high stability because the acetazolamide have electron with drawing group (sulfonamide) makes $\text{N}\equiv\text{N}$ group more positive charge and 8-Hydroxyquinoline have electron donating group makes ring very active so easy formation of high stability azo-coupling reaction between acetazolamide and 8-hydroxyquinoline[26]. The proposed mechanism of reaction illustrated in Scheme.II.

Pharmaceutical applications

The proposed methods approach and cloud point extraction were applied successfully for determination of
three concentrations form Acetazolamide in (Cidamex and Diamox 250mg) tablets as shown in the Table.3, after studying the effect of additives by adding separately excess amounts about (10:1) from additives to (40 µg.mL⁻¹) acetazolamide solutions under optimum reaction conditions followed in the calibration curves. As shown in a Table. 2.

**Table 2. Determination of (40 µg.mL⁻¹) from Acetazolamide in the presence of additives**

<table>
<thead>
<tr>
<th>Excipient</th>
<th>Acetazolamide (40 µg.mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conce found µg.mL⁻¹</td>
</tr>
<tr>
<td>Pvp</td>
<td>39.83</td>
</tr>
<tr>
<td>Lactose</td>
<td>40.05</td>
</tr>
<tr>
<td>Starch</td>
<td>39.69</td>
</tr>
<tr>
<td>Mg stearate</td>
<td>40.07</td>
</tr>
</tbody>
</table>

*Average of five determinations

**Table 3. Application of proposed methods on pharmaceutical preparation for Acetazolamide**

Evaluate the results of the proposed methods

The standard method for estimation Acetazolamide in the British pharmacopoeia was applied for determination of acetazolamide in pure drug and Pharmaceutical Preparations. The results of standard method comparison with proposed methods approach and cloud point extraction (F and t test value). The results summarized in the Table.4. Shown no significant differences between the two methods.

**Table 4. Application of F, and t test for comparison between proposed and standard methods**

<table>
<thead>
<tr>
<th>Pharmaceutical preparation</th>
<th>Proposed method</th>
<th>Standard method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rec.*%</td>
<td>Rec.*%</td>
</tr>
<tr>
<td>Pure Acetazolamide</td>
<td>99.37</td>
<td>0.0009</td>
</tr>
<tr>
<td>Cidamex</td>
<td>99.43</td>
<td>0.0081</td>
</tr>
<tr>
<td>Diamox</td>
<td>99.23</td>
<td>0.0100</td>
</tr>
</tbody>
</table>

F-Value (experimental) = 0.1000, Critical F-Value (19.000)
t-Value (experimental) = -0.9944 , Critical t-Value (2.776)

<table>
<thead>
<tr>
<th>Pharmaceutical preparation</th>
<th>Proposed method</th>
<th>Standard method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rec.*%</td>
<td>Rec.*%</td>
</tr>
<tr>
<td>Pure Acetazolamide</td>
<td>99.20</td>
<td>0.0120</td>
</tr>
<tr>
<td>Cidamex</td>
<td>99.37</td>
<td>0.0025</td>
</tr>
<tr>
<td>Diamox</td>
<td>99.39</td>
<td>0.0049</td>
</tr>
</tbody>
</table>

F-Value (experimental) = 0.0979, Critical F-Value (19.000)
t-Value (experimental) = -1.0990, Critical t-Value (2.776)
Conclusions

A simple, rapid, sensitive and new selective Spectrophotometric methods have been developed, not affected by excipients, successfully applied for determination of trace amounts of acetazolamide drug in pure and pharmaceutical formulations based on basic hydrolysis of acetazolamide and coupling the hydrolysis product with 8-hydroxyquinoline reagent depending on the diazonium coupling reaction.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Science, Babylon University, Iraq and all experiments were carried out in accordance with approved guidelines.

References


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Abstract
Successful osseointegration of dental implants depends on many factors, including implant stability, bone quality, implant surface condition, and implant materials. Implant surface is coated using different techniques to enhance osseointegration. Sol–gel technique is one of the modern and easy techniques in the production of more bioactive hydroxyapatite (HA).

In this study, nano-HA and chitosan were used to coat titanium disc via the sol–gel technique.

Materials and Method: The nano-HA/chitosan composite was prepared using calcium nitride and phosphorus pentoxide solutions. The solutions were drop wisely mixed on a magnetic stirrer. Then, 3 mg of chitosan was added to the mixture, and the mixture was stirred for 15 h. Titanium samples were dip coated in the mixture of chitosan/HA on the stirrer at slow speed for 90 min to precipitate the coating layer. Afterward, the samples were removed and dried in a hot air oven. They were sintered at 400 °C for 1 h. X-ray diffraction (XRD), light microscopy, scanning electron microscopy (SEM), energy-dispersive x-ray spectroscopy (EDX), FTIR analysis, and atomic force microscopy (AFM) were used to analyze the surface and thickness.

The results of XRD test showed that HA particles had sharp diffraction peaks, indicating high crystallinity of the structure due to the sol–gel preparation. Results of optical light microscopy showed that the coated layer was fairly distributed on titanium samples. SEM results of the coated samples showed different surface features, which present a roughness with the appearance of a crystal pool with irregular accumulation of small, spherical-like granules. EDX analysis showed the presence of ions that comprised HA. AFM analysis of the coated sample indicated peaks and projections with average surface nano-roughness (10.1 nm) and grain size (2022–2057 nm). FTIR analysis showed the presence of a band associated with HA particles and amide groups associated with chitosan. The average of thickness readings was 60 μm.

Keywords: dental implant, dip coating, sol–gel, chitosan, hydroxyapatite, nanoparticles.

Introduction
Osseointegration is the apparent direct attachment or connection of osseous tissue to an inert, alloplastic material without intervening fibrous connective tissue; it is the process and resultant apparent direct connection of an exogenous material’s surface and the host bone tissues. Several factors affect the success of osseointegration. One of these factors is implant stability, which is composed of two types: primary stability, which occurs due to mechanical support of implant to the surrounding bone immediately after implant insertion and secondary stability, which occurs due to biological support after bone regeneration and remodeling. Other factors include bone quality, surgical technique, implant surface condition, implant material, and implant surface. The type of material that covers the implant surface is an important factor in enhancing osseointegration for initial stability. Therefore, several attempts have been made to enhance osseointegration at the bone/implant interface by making various coatings to support the main implant. Titanium and its alloy are commonly
used in the fabrication of artificial joints and tooth roots due to their excellent mechanical properties that closely resemble natural bone \(^6\). Hydroxyapatite (HA) is a commonly used material for coating implant surface because it resembles human bone in its chemical and crystallographic structures, and it has the advantage of acting as a barrier that reduces the release of metallic ions \(^7\). The use of nanophase particles of HA increases the number of particles and surface areas, thereby increasing the reactivity of HA and enhancing bioactivity \(^8\). The sol–gel technique is one of the modern techniques for preparing HA to enhance the surface bioactivity of implant and increase bone attachment \(^9\). Anuar et al. (2013) produced HA nanoparticles using the sol–gel method \(^10\). Taherian et al. (2014) studied the effects of different sol–gel synthesis processes on HA nanopowders \(^11\). Chitosan has many medical applications because of its biocompatibility and non-toxicity \(^12\). It can clot blood to aid in hemostatic action, and it can stimulate macrophages to initiate collagen deposition, which accelerates the healing process \(^13\).

**Materials and Methods**

**Preparation of sol–gel**

Nano-HA was prepared using the sol–gel technique according to a previously reported method with slight modifications \(^16\).

Preparation of first solution: An electronic balance (accuracy 0.0001 g, Germany) was used to weigh 10.78 g of calcium nitride (Ca(NO\(_3\))\(_2\)). Ca(NO\(_3\))\(_2\) was then mixed with 125 ml of ethyl alcohol on a magnetic stirrer (SH – 3, England). Stirring was performed for 1 hour until the solution was completely dissolved.

Preparation of second solution: The solution was prepared by mixing 5 g of phosphorus pentoxide (P2O5) with 125 ml of ethyl alcohol on a magnetic stirrer for 1 hour until completely dissolved.

After the two solutions were mixed, Ca(NO\(_3\))\(_2\) was added drop wise on P2O5 for 2 h under continuous stirring. Next, 5 g of potassium hydroxide (KOH) was added to the mixture to complete the reaction. Finally, 3 g of chitosan was added to the mixture, and the mixture was stirred for 15 h.

**Sample preparation**

Circular commercially pure titanium (Cp-Ti) discs of 10 mm diameter and 2.5 mm thickness were prepared as follows: Cp-Ti rod grade 2 (Orotig S.r.l., Italy) was cut and mirror polished using 500 micron roughness silicon carbide for 15 min. The discs were then cleaned in an ultrasonic bath with ≥99.8% ethanol to eliminate debris and contamination. After 15 min, the cleaning process was completed; the discs were washed with distilled water for 10 min and left at room temperature to dry \(^17\).

**Dip coating procedure**

The titanium samples were dip coated in the mixture of chitosan/HA on the stirrer at slow speed for 90 min to precipitate the coating layer. Then, the samples were removed and dried in a hot air oven (200 °C) (IMS/406, France).

**Heat treatment (sintering)**

Sintering was performed using a tube furnace (Carbolite Type MTF 12/38A. BAMFORD, England). A pilot study was performed using the three heating methods as follows:

A. Coated at the sol stage and sintered at 400 °C for 1 h
B. Coated at the gel stage and sintered at 125 °C for 1 h
C. coated by gel stage and sintering at 400 °C for one hour.

Examination of specimens after sintering by X-ray diffraction analysis revealed that samples of titanium were coated with nano-hydroxyl apatite and chitosan at the gel stage and sintered at 400°C for one hour showed the best results.

**Analysis of coating layer**

The coated surface of the specimens was analyzed as follows:

**Thickness measurement:**

The thickness of the coating layer was measured by using microprocess coating thickness gauge (TF-C-UVIS-SR, USA). Three readings were obtained, and their average was 60 μm.

**XRD analysis:**

The Shimadzu XRD-6000 (Japan) diffractometer was used to provide information about the chemical composition and crystallographic structure of materials.
The indexing of peaks was based on the Joint Committee of Powder Diffraction Standards (JCPDS).

Optical microscopy examination (light microscopy):

The features of the coating surface were examined by using a light microscope (Nikon Eclipse ME 600 L/441002, Japan) and compared with those of the control sample. The analysis was performed by using a digital camera (DXM1200F) connected to computer with a software program for analysis.

Structural surface characterization by SEM:

- Surface analysis: The surface morphology and topographical characteristics of the coated sample were determined by SEM (TESCAN Vega 111, Czech Republic).

- Material characterization: Material characterization was performed using EDS, and SEM was used for chemical analysis of the sample, depending on the interaction and excitation of X-ray.

AFM:

AFM was used for analysis of surface roughness. It is an advanced type of profile meter used to produce topographical image where a very sharp and inert tip was scanned over the surface of the coated sample.

**Results**

**Thickness measurement**

The thickness of the coated layer was measured using a microprocess thickness gauge. The thickness of the coating film increased with time. The average of three readings was 60 μm.

![Graph showing thickness measurement](image)

**Fig.1 Thickness of nano-HA and chitosan coating layer at 0.5, 1, 2, and 3 min**

**X-ray diffraction analysis:**

According to standard pattern of (JCPDS09 – 0432) the peaks of HA were indexed according to, as shown in Fig. (2) a and b. the Stoichiometric of HA particles displayed peaks with sharp diffraction that representing high crystallinity of the structure. By XRD, There was no identification of impurity phase.
FTIR analysis

FTIR analysis results show the coated surface with peaks of HA and chitosan. In Fig. 3.2, FTIR spectra illustrated an OH\_ band at 3569 cm\(^{-1}\) and PO\(_4\)\_4 bands at 472, 565, 603, and 1032 cm\(^{-1}\) associated with HA. The sharp peak (1637 cm\(^{-1}\)) and broad bands for adsorbed water (3000–3500 cm\(^{-1}\)) prove water absorption due to the high specific surface that precipitated powders usually have. The CO\(_2\)\_3 groups, which can substitute both PO\(_4\)\_4 and OH\_ ions in the HA structure, appeared as 1461, 1423, and 875 cm\(^{-1}\) wave numbers. The presence of amide groups I, II, and III at 3500–3700, 1630–1690, and 1735–1750 cm\(^{-1}\), respectively, corresponded with chitosan particles.
Light microscopy examination

Light microscopy examinations revealed the surface image of coated and non-coated titanium samples at 250 μm magnification, as shown in Fig. 3.4; lines appeared on the uncoated samples, which resulted from machining, whereas the coated sample showed homogenous coating layer without cracks.

![Fig. 4 Light microscopy examination of coated and uncoated samples](image)

AFM analysis

Figure 3.4 shows the surface features of coated surface with mixture of HA and chitosan. Peaks and projections with average surface nano-roughness of 10.1 nm and grain size (2022–2057 nm). Table 1 lists the roughness values obtained from AFM images.

![Fig. 5 AFM image for the coated surface with mixture of HA and chitosan](image)

Table 1: Roughness values (nm) obtained from AFM images for all test groups

<table>
<thead>
<tr>
<th>group</th>
<th>Average diameter of grain</th>
<th>Roughness average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (uncoated)</td>
<td>222.15 nm</td>
<td>7.37 nm</td>
</tr>
<tr>
<td>Coated sample</td>
<td>70.62 nm</td>
<td>10.1 nm</td>
</tr>
</tbody>
</table>
**Discussion**

HA has hydroxyl groups that can promote nucleation of phosphate and calcium, which enhances bone formation after implantation \(^{18}\). Chitosan has high viscosity in solution \(^{19}\), so it was hybridized with sol–gel HA to enhance apatite formation \(^{20}\). Therefore, the present study used a HA/ chitosan composite.

HA was successfully coated with titanium via HA sol–gel process. The sol–gel process produces more bioactive HA compared with other techniques \(^{21}\).

Dip coating technique was used to successfully implant HA on titanium \(^{22}\). Dip coating/sol–gel technique has the advantages of controlling coating thickness \(^{23}\).

SEM of the coated film revealed different surface features that appeared as rough surfaces with homogenous and crack-free distribution of the particles, as well as the appearance of crystal pool with accumulation of small, spherical-like granules. This manner of accumulation is one of the properties of HA prepared via sol–gel method \(^{24}\). This result is considered as good property, which indicates wide area of contact with living tissue, and is consistent with the results of A. Balamurugan et al., 2002 \(^{25}\), who studied the biomedical applications of sol–gel HA. Figure 3.6 shows the existence of O, P, and Ca elements, which are related to HA nanoparticles. The EDX results ensured that the coating layer was evenly distributed with no elements being concentrated in some areas more than others \(^{26}\).

FTIR spectra illustrated an OH_ band at 3569 cm\(^{-1}\) and PO\(_3\_4\) bands at 472, 565, 603, and 1032 cm\(^{-1}\) associated with HA. The sharp peak (1637 cm\(^{-1}\)) and broad bands for adsorbed water (3000–3500 cm\(^{-1}\)) prove water absorption because of the high specific surface area that precipitated powders usually have. The CO\(_2\_3\) groups, which can substitute both PO\(_3\_4\) and OH_ ions in the HA structure, appeared at 1461, 1423, and 875 cm\(^{-1}\) wave numbers. The presence of amide groups I, II, and III at 3500–3700, 1630–1690, and 1735–1750 cm\(^{-1}\), respectively, corresponded with chitosan particles. The result ensured the formation of HA by the appearance of phosphate bands, at 3500–3700, 1630–1690, and 1735–1750 cm\(^{-1}\), respectively, corresponded with chitosan particles. The result ensured the formation of HA by the appearance of phosphate bands \(^{27}\).

**Conclusion**

Sol – gel technique was successful method to prepare composite of nano HA/chitosan that showed uniform and homogenous thickness of coatings that improved surface properties of titanium implant when compared with uncoated surface. The use of dip coating procedure was benefit to achieve thin, homogenous and crack free coating film on CP – Ti surface.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of Dentistry, Uruk University, Baghdad, Iraq and all experiments were carried out in accordance with approved guidelines.

**Reference**

1. Keith J. Ferro, Editor and Chairman, Glossary of Prosthodontic Terms Committee, journal of prosthodontic dentistry. 2017
7. Roxana family, Mehran Solati-Hashjin, Shahram Namjoy Nik and : Surface modification for titanium implants by hydroxyapatite nanocomposite,


In this paper, a cellulose paper was impregnated with silver nanoparticles (AgNPs) for the purpose of removing Enterococcus faecalis from drinking water. AgNPs papers were prepared by chemical reduction of silver nitrate (AgNO3) with various concentrations (0.005 M, 0.01 M, 0.015 M, and 0.025 M) using sodium borohydride (NaBH4) as a reducing agent. Two ratios of NaBH4/AgNO3 of 2:1 and 10:1 were used to show the effect of reduction on the formation and removal efficiencies of AgNPs. AgNPs papers were characterized using SEM and TEM. TEM images showed that the silver nanoparticles size in the papers varies from 1.3 to 75 nm.

**Keywords:** Enterococcus Faecalis, Drinking Water, Silver Nanoparticles.

**Introduction**

Disinfection of potable water is the specialized treatment for destruction or removal of organisms capable of causing disease; it should not be confused with sterilization, which is the destruction or removal of all life. Although disinfection methods currently used in drinking water treatment can effectively control microbial pathogens, researches in the past few decades have revealed a dilemma between effective disinfection and formation of harmful disinfection byproducts (DBPs). Three categories of human enteric pathogens are of concern in drinking water: bacteria, viruses, and amebic cysts. Disinfection must be capable of destroying all three. Destruction or removal of these organisms is essential in providing a safe potable water supply. Some bacteria, viruses, protozoa, and larger organisms ingested from contaminated water cause diseases varying from mild illnesses to life-threatening. The Enterococcus genus is placed in the Enterococcaceae family and consists of species that occur in human and animal gastro-intestinal (GI) tracts, as well as in the guts of insects traditional fermented food and dairy products, and in various environments including plants, soil and water. Enterococcus faecalis is a non-spore-forming, fermentative, facultatively anaerobic, Gram-positive coccus. Enterococcus faecalis cells are ovoid and 0.5 to 1 μm in diameter. They occur singly, in pairs, or in short chains, and are frequently elongated in the direction of the chain. They typically have an optimum growth temperature of 35°C and a growth range from 10 to 45°C. They currently rank among the most prevalent multidrug resistant hospital pathogens worldwide as the third most commonly isolated healthcare pathogen, and are capable of causing a variety of infections including endocarditis, sepsis, surgical wound infections, and urinary tract infections. The organism has the natural ability to acquire, accumulate and share extrachromosomal elements encoding virulence traits, which help to colonize, compete with other bacteria, resist host defense mechanisms and produce pathological changes directly through production of toxins or indirectly through induction of inflammation. Nanotechnology and its application is one of the rapidly developing sciences. Silver nanoparticles have proved to be most effective as it has good antimicrobial efficacy against bacteria, viruses and other eukaryotic microorganisms.

**Experimental Procedure**

**Sampling**

The samples were taking from Shatt al-Hilla, at Al-Hilla city/Iraq and during the period (November 2018 – March 2019). 500 ml of water as grabbed and kept in precleared plastic bottle. The samples were analyzed immediately to prevent any change in their quality that
may occur.

**Preparation of AgNPs papers**

A (10 cm * 10 cm * 0.8 mm) off-white paper, 100% alpha cellulose was used to be embedded with silver nanoparticles. AgNPs papers were prepared by in situ reduction of AgNO₃ with various concentrations (0.005 M, 0.01 M, 0.025 M and 0.05 M) with two reduction ratio of 2:1 and 10:1. Each paper was soaked in 40 ml of AgNO₃ solution for 30 minutes, then it was washed with ethanol for 1 minute to remove the excess Ag ions which not absorbed by the paper. To form AgNPs, the paper was placed in 40 ml of NaBH₄ solution for 1 hr. After that, the paper was soaked in de-ionized water for 30 minutes. Then the paper was dried in the oven at 60 °C for 2.5 hrs.

![Fig. 1: (a) AgNPs paper during preparation with NaBH₄/AgNO₃ ratio of 2:1. (b) AgNPs paper during preparation with NaBH₄/AgNO₃ ratio of 10:1.](image)

**Characterization**

The synthesized AgNPs papers were characterized by Scanning Electron Microscopy (SEM), type Quanta 450 available at the University of Babylon/College of Pharmacy and Transmission Electron Microscopy (TEM) available at Al-Nahrain University/College of Medicine.

**Acid Digestion**

To determine the silver content in the AgNPs paper, an acid digestion of the paper was performed and then analyzes the amount of dissolved silver with an Atomic Absorption Spectrometer (AAS) (AA320N) available at the University of Babylon/College of Material Engineering. Approximately a 100 mg of the dried AgNPs paper was reacted with 5 ml of nitric acid (HNO₃) and 5 ml of water. The mixture was boiled until the paper was disintegrated. 5 ml of 30% hydrogen peroxide (H₂O₂) was added to the mixture to assist in the complete oxidation of the organic matter to release additional metals into the solution. The mixture was boiled again and left to be cooled, then filtered through a filter paper and then diluted by adding a 100 ml of water. The diluted mixture was tested for silver content using an AAS.

**Microbiological Test**

The raw water samples were cultured using serial dilutions method, 1 ml of the sample was diluted in 9 ml of distilled water (1:10 dilution). 1 ml of 1:10 dilution mixed with 9 ml of distilled water (1:100 dilution), etc. the filtered water samples were cultured without dilution. 0.1 ml of each sample was spread over a media plate and then the plates were incubated in 37 °C for 48 hrs in an incubator (LIB-030M).
Results and Discussion

Paper Characterization

The AgNPs papers were characterized by SEM and TEM. Fig. 2 represents the images obtained by SEM to show the presence of AgNPs in paper fibers. Fig. 3 represents the images obtained by TEM to determine the particles sizes of AgNPs. Table 1 represents the particles sizes of AgNPs obtained by TEM test.

![Fig. 2: Images obtained from SEM. (a) 2:1 NaBH4/AgNO3 ratio. (b) 10:1 NaBH4/AgNO3 ratio.](image)

![Fig. 3: TEM images. (a) 2:1 NaBH4/AgNO3 ratio. (b) 10:1 NaBH4/AgNO3 ratio.](image)

<table>
<thead>
<tr>
<th>AgNO3 concentration, M</th>
<th>Nanoparticle Size Range ,nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2:1 NaBH4/AgNO3 ratio</td>
</tr>
<tr>
<td>0.005</td>
<td>6.86 - 75</td>
</tr>
<tr>
<td>0.01</td>
<td>3 – 69.26</td>
</tr>
<tr>
<td>0.025</td>
<td>1.414 – 32.802</td>
</tr>
<tr>
<td>0.05</td>
<td>2 – 21.84</td>
</tr>
</tbody>
</table>
TEM images showed that an excess of sodium borohydride reductant (10:1 ratio of sodium borohydride to silver nitrate) gave more uniform and smaller nanoparticles.

**Acid Digestion**

Acid digestion was performed to determine the silver content of the paper. The results were obtained by using AAS (AA320N). Table 2 shows the results of the AAS test.

**Table 2: Silver content of each paper**

<table>
<thead>
<tr>
<th>AgNO3 concentration, M</th>
<th>Silver content (mg Ag/g of dried paper)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2:1 NaBH4/AgNO3 ratio</td>
</tr>
<tr>
<td>0.005</td>
<td>3.9584</td>
</tr>
<tr>
<td>0.01</td>
<td>4.4765</td>
</tr>
<tr>
<td>0.025</td>
<td>6.3268</td>
</tr>
<tr>
<td>0.05</td>
<td>7.8669</td>
</tr>
</tbody>
</table>

The acid digestion of AgNPs papers showed silver content ranging from 3.9 to 8.7 mg Ag per dry gram of paper. The increase in silver content of the paper correlates with the increase in precursor silver ion concentration of the solution in which the papers were soaked, prior to reduction. For the same concentration of AgNO3, the NaBH4/AgNO3 ratio of 10:1 resulted in more silver content than 2:1 ratio.

**Bactericidal Effectiveness of AgNPs papers**

Fig. 4 shows the effect of the silver content in the AgNPs paper on the removal efficiency of Enterococcus faecalis of filtered water samples and raw water samples with a NaBH4/AgNO3 ratio of 2:1 and 10:1 respectively.

![Graph](image)

(a) 

(b)

Fig. 4: Effect of silver content on the removal efficiency of Enterococcus faecalis of raw water samples with a: (a) NaBH4/AgNO3 ratio of 2:1. (b) NaBH4/AgNO3 ratio of 10:1.

Fig. 4 shows that removal efficiency Enterococcus Faecalis of the raw water samples for both ratios ranges from 99.9 % to 100% for all silver contents.
Analysis of silver content in The Effluent

Due to possible human health effects from silver exposure, the silver content in the effluent water was analyzed by AAS. Table 4 represents relationship between the silver content in the paper and silver release in the effluent.

Table 4: The relationship between the silver content in the papers and silver in the effluent water.

<table>
<thead>
<tr>
<th>AgNO3 concentration, M</th>
<th>NaBH4/AgNO3 ratio</th>
<th>Silver Content in the Effluent, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.005</td>
<td>2:1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>10:1</td>
<td>0</td>
</tr>
<tr>
<td>0.01</td>
<td>2:1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>10:1</td>
<td>0</td>
</tr>
<tr>
<td>0.025</td>
<td>2:1</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td>10:1</td>
<td>0.043</td>
</tr>
<tr>
<td>0.05</td>
<td>2:1</td>
<td>0.043</td>
</tr>
<tr>
<td></td>
<td>10:1</td>
<td>0.082</td>
</tr>
</tbody>
</table>

As shown in Table 4, the average silver content in the effluent water for the three replicates range from 0 to 0.082 which meets the United States Environmental Protection Agency (US-EPA) guideline for drinking water of less than 0.1 mg/L [EPA, 2018]. This was due to the stability of silver nanoparticle in the cellulose paper. Sodium borohydride acts not only a reducing agent but also as an ion stabilizer, which prevents silver ions from aggregation. Moreover, hydroxyl and ether groups in the cellulose fiber play an important role in the stabilization of metal nanoparticles.

Conclusions

Silver nanoparticles used in this study were well dispersed and stabilized on the paper fibers. Chemical reduction of AgNO3 by using NaBH4 as a reducing agent resulted in spherical silver nanoparticles. The NaBH4/AgNO3 ratio of 10:1 resulted in smaller sizes of silver nanoparticle and more silver content than the ratio of 2:1 for the same AgNO3 concentration. (99.9-100)% inhibition of Enterococcus Faecalis was obtained with all the concentrations of AgNO3 and NaBH4 and for both NaBH4/AgNO3 ratios.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Water Resources Engineering/ Al-Qassim Green University and all experiments were carried out in accordance with approved guidelines.

References


Assessment of the Correlation between the Salivary Flow Rate and Dental Caries Experience among Children with β-Thalassemia Major

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Abstract

Thalassemia constitutes a group of congenital blood disorders which characterized by a defect in synthesis of one or more globin chains of human hemoglobin molecule and the resultant microcytosis and hypochromia of the RBCs. It is of two main divisions, α- and β-thalassemia and several other subdivisions. The study group composed of 40 patient years previously diagnosed with β-thalassemia major attending the thalassemia center in Thi-Qar province/Iraq. The control group, matching the age and sex of the study group, and consisted from 40 child selected from a number of primary schools. Unstimulated salivary samples was taken from each subjects under standardized conditions. The mean value of flow rate of saliva was lower among β-thalassemias (0.466±0.024) than for controls (0.829±0.048). The (mean±SE) for the primary teeth (dmfs) in β-thalassemias (1.450±0.324) was higher than that for controls (1.250±0.808), this difference was not significant (P> 0.05). The (mean±SE) of the caries experience (DMFs) in β-thalassemias (6.850±0.782) was higher than for the control group (3.600±0.489).

Keywords: β-Thalassemia major, Salivary flow rate, Dental caries experience (dmfs/DMFs).

Introduction

Thalassemia is a very worldwide common autosomal and recessive genetic disorder with a large geographical incidence difference, it is a so severe and incurable disease because prevention is the only way to evade the disease [1]. Thalassaemias are caused by markdown or complete absent in the synthesis of one or more of the globin chains that constitute the hemoglobin (Hb) unit [2,3]. About 60,000 to 70,000 newly born children are born yearly with a severe form of thalassaemias around the world [4], and unfortunately most affected children are those who born in areas of low sources of income [5]. β-Thalassemia major is the most severe type of thalassemia and occurs due to a defect in the synthesis of β-globin chain [6]. Beta- homotetramers in α-thalassemia are more stable than alpha- homotetramers in β-thalassemia; therefore, in beta thalassemia α-homotetramers tend to precipitate priorly in the RBCs life span, producing marked RBCs haemolysis [7]. Few of the pro-erythroblasts beginning their maturation and can survive [8,9], the resultant few RBCs will bear an inclusion bodies that identified in the spleen, shortening the RBC lifespan and producing severe haemolytic anaemia [10]. The produced anaemia stimulates the production of erythropoietin stimulating hormone from the kidney and liver with a compensatory erythroid hyperplasia, but the marrow response is disrupted by ineffective erythropoiesis. [11]. Later on, massive bone marrow expansion will happened and the end result is skeletal deformities[12]. In thalassemias, high caries index, pallor of oral mucosa, atrophic glossitis, sialadenitis, retained deciduous teeth, shortened and spiked roots of teeth and thinning of the lamina dura could be seen [13]. Oral health, on the other hand, can also affect child’s personality, knowledge, and social relationships [14]. Dental caries is a chronic disease resulted from a complex intercommunication of a mass of cariogenic oral microorganisms which grow on the teeth surfaces within the dental [15]. Dental caries is widely spread and can affecting all ages. The experience of dental caries could

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be modified by several important factors, including diet, age, gender, socioeconomic level and some medicines [16]. Saliva is a heterogeneous mixture of fluids mainly produced via the major and minor salivary glands and contains oral bacteria and food debris from the gingival crevicular fluid [17,18]. Saliva have an important role in the maintenance of a healthy oral environment through a variety of physicochemical and biological properties. Unstimulated (resting) saliva includes secretions that enter the mouth without any exogenous stimuli [19,20]. Flow rate is the most important salivary parameter affecting oral health status and it represents the rate of salivary secretion and it is expressed in milliliters per minute [21]. Flow rate is very important protective factor against dental caries throughout its washing and buffering effect [22], any minimization in the normal salivary flow rate can results in establishment of dental caries [23]. Al-Jobouri and Al-Casey [24] and Kataria et al. [25]

**Materials and Method**

The total sample included in this study was (80) subjects. The study group was consisted of a (40) child suffering from β-thalassemia major and aged (11-12 years old). The control group, the non-thalassemic children, matching the age and gender of the study group, and consisted of a (40) child selected from the primary schools. An ethical approval was firstly obtained from the Ministry of Health and the Ministry of Education to perform the clinical examination and laboratory biochemical analysis. Also, a written consent form as well as patient information sheet were provided to each participant for gaining the acceptance of the child’s parents or his/her caregiver. Children having other diseases whose known to affect the experience of dental caries or the characteristics of saliva such as diabetes mellitus, were excluded. The oral examination was performed under standardized conditions of oral health surveys (WHO 1997). During examination, a suitable chair is used with noticing that it supports the head of the child. Clinical examination was performed using plane mouth mirror and dental probe. The reported caries experience was based on the criteria suggested by Manjie et al. [26]. Unstimulated salivary samples was taken from each subjects under standardized conditions suggested by Navazesh and Kumar [27]. Statistical analyses were done using SPSS computer programme, version 21. Descriptive statistics including (the mean and SE) of each clinical variable were determined for all the subjects. Student t-test was used to compare the caries experience between the study and control groups. Pearson’s correlation coefficient (r) was used to assess and compare the correlations among the variables. (P> 0.05) was considered statistically not significant. (P≤ 0.05) was considered statistically significant and (P≤ 0.01) was considered to be statistically highly significant.

**Results**

Table (1) illustrates the results showed that the (mean±SE) in β-thalassemias (1.450±0.324) was higher than that for the control group (1.250±0.808), This difference was not significant (P> 0.05).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Study</th>
<th>Control</th>
<th>t-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>dmfs</td>
<td>1.45</td>
<td>0.324</td>
<td>1.250</td>
<td>0.808</td>
</tr>
</tbody>
</table>

* Not significant difference (P> 0.05).

Table (2) illustrates the results showed that the (mean±SE) value in β-thalassemias (6.850±0.782) was higher than that for the control group (3.600±0.489). This difference was highly significant (P≤ 0.01).
Table (2): Dental caries experience by surfaces in permanent dentition among β-thalassemias and their controls.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Study</th>
<th>Control</th>
<th>t-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>DMFs</td>
<td>6.85</td>
<td>0.782</td>
<td>3.600</td>
<td>0.489</td>
</tr>
</tbody>
</table>

** Highly significant difference (P ≤ 0.01)

Table (3): Results showed that the salivary flow rate among β-thalassemias was lower among β-thalasemia patients than for their control subjects. This difference was highly significant (P ≤ 0.01).

Table (3): Salivary flow rate among thalassemia patients and their controls.

<table>
<thead>
<tr>
<th>Study</th>
<th>Control</th>
<th>t-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.466</td>
<td>0.024</td>
<td>0.829</td>
</tr>
</tbody>
</table>

** Highly significant difference (P ≤ 0.01)

Table (4) In primary dentition, results showed a weak negative, not significant correlation between the salivary flow rate and (dmfs) index in the study and control groups. In permanent dentition, results showed a weak negative, not significant correlation between the salivary flow rate and (DMFs) index in the study and control groups.

Table (4): Correlation coefficients of the salivary flow rate and the dental caries experience among thalassemia patients and their controls.

*NS = Not significant

Discussion

In the present study, the mean value of salivary flow rate was founded to be lower among β-thalassemias (0.466±0.024) than in their control subjects (0.829±0.048). This result was also documented previously by Norri [28] and by Al-Jobouri and Al-Casey [24]. This minimization in the salivary flow rate among the study group might be attributed to the fact that the salivary glands function in thalassemic patients can be affected directly by the excessive iron deposits, and the resultant painful inflammation of the salivary glands, with either regular or diminished salivary flow [23]. However, Siamopoulou et al [29] concluded in their study that the difference in salivary flow rate between the two groups is not significant. These results were in disagreement with Luglie et al. [30] and Greenberg et al.
whose showed in their studies that salivary flow rate was similar in both study and control groups.

The present study also concluded that the mean value of caries experience by surfaces in primary dentition (dmfs) among β-thalassemias was higher than that recorded in their controls. This result was corresponding with Gomber et al. [32], Kaur et al. [33], Dhote et al. [34]. On the contrary, this result was disagree with Scutellori et al. [35], Qureshi et al. [36] and Arora et al. [37] who were concluded that there is no difference between the two groups.

Furthermore, the results of the present study revealed that the mean value of caries experience by surfaces in permanent dentition (DMFs) among β-thalassemias was higher than that recorded in their controls. This results was in agreement with Hattab et al. [38], Al-Raheem et al. [39] and Al-Hadithi [40], while it was in disagreement with the results of Scutellori et al. [35] and Arora et al. [37] who were founded a similar mean value between the two groups.

In present study, there was a negative correlation between salivary flow rate and caries experience. Salivary flow rate may play an important role in relation to dental caries in which the flow rate of saliva exert cleansing activity which is critical in the clearance of food remnants and bacteria [41]. It was documented that the caries experience is obviously increased when salivary flow rate is stunted and this fact sign that the chronic decrease in flow rate is a risk factor for dental caries initiation and progression [42]. In thalassemic patients, reduced salivary flow rate can help in colonization of cariogenic S. mutans, which may have a role in the higher caries experience. Al-Zaidi [43] has documented an indubitable correlation between oral mutans streptococci and dental caries in β-thalassemia patients.

However, the higher caries experience in β-thalassemias than the normal controls can be related to factors other than a reduced salivary flow rate. This could be explained on the idea that these patients are less concern with their oral health, their parents are more worried about the serious physical condition, they paying less care to the oral health care, and seeking professional oral care just when the child experience severe pain [23, 44].

Al-Wahadni [45] and Gomber and Dewan [32] concluded in their studies that skeletal changes as enlargement in maxillary arch that occur in thalassemias in could result in protrusion of anterior segment, increased space between upper and lower teeth, over-bite or open-bite which all could predispose to dental caries.

**Conclusion**

Dental caries experience was higher among beta thalassemias compared to the control group. The salivary flow rate among β-thalassemias was lower among β-thalassemia patients than for their control subjects. The reduced salivary flow rate detected in β-thalassemia major patients could be considered as a modifying factor for the increased experience of dental caries in these patients.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of Dentistry/Univ. of Baghdad, Iraq and all experiments were carried out in accordance with approved guidelines.

**References**


Determine Effect of Carbothera Therapy on Foot and Leg Ulceration for Diabetic Patients in Endocrine and Diabetic Center at Al-Nasiriya City

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Abstract

Background: Diabetic foot is a serious complication of diabetes that aggravates the patient’s condition whilst also having significant socioeconomic impact. Increasing physicians’ awareness and hence their ability to identify the «foot at risk,» along with proper foot care, may prevent diabetic foot ulceration and thus reduce the risk of amputation.

Objectives: 1. Determine the socio demographic data on diabetic foot and leg ulceration
2. Determine the relationship of socio demographic data on diabetic foot and leg ulceration
3. Determine the Effecteness of Carbothera therapy upon diabetic foot and leg ulceration improvement

Methodology: A descriptive study was conducted on a sample consisting of (50) patients is having diabetic foot and leg ulceration who were selected randomly from Endocrine and Diabetic Center in Nasiriya City. Diabetic patient who have diabetic foot and leg ulceration, carried out in Nasiriya city / endocrine and diabetic Center starting from January/ 2019 to July /2019

Results: 40% the age between 60-69 years, males was 68%, the patients residing in the city (urban) by 66%,78% of them consider that their monthly income is not enough, infected with diabetes For diabetes for a period of 10 years and more is 76%, the average age of patients was 59 years, patients discovered diabetes disease was 66% , improvement in their health through the carbothera therapy 66% indicated a positive effect on the patients, a significant mean relationship at level of 8% between the urban and rural environment variable with improved carbothera therapy indicates that therapy is more effective when the patient has a more urban than rural. In addition, a very weak and statistically insignificant relationship between the patients is other demographic data and the improvement of carbothera therapy

Keywords: Diabetic, Carbothera therapy, foot and leg ulceration

Introduction

Diabetic foot and leg ulceration is one of the most significant and devastating complications of diabetes, and is defined as a foot affected by ulceration that is associated with neuropathy and/or peripheral arterial disease of the lower limb in a patient with diabetes. The prevalence of diabetic foot ulceration in the diabetic population is 4–10%; the condition is more frequent in older patients 1-3. It is estimated that about 5% of all patients with diabetes present with a history of foot ulceration, while the lifetime risk of diabetic patients developing this complication is 15% 1. The majority (60–80%) of foot ulcers will heal, while 10–15% of them will remain active, and 5–24% of them will finally lead to limb amputation within a period of 6–18 months after the first evaluation. Neuropathic wounds are more likely to heal over a period of 20 weeks, while neuro
ischemic ulcers take longer and will more often lead to limb amputation \(^4\). It has been found that 40–70\% of all no traumatic amputations of the lower limbs occur in patients with diabetes \(^5\). Furthermore, many studies have reported that foot ulcers precede approximately 85\% of all amputations performed in diabetic patients \(^5\). The risk of foot ulceration and limb amputation increases with age and the duration of diabetes \(^6,7\). The prevention of diabetic foot is crucial, considering the negative impact on a patient’s quality of life and the associated economic burden on the healthcare system \(^8\). Diabetic foot ulceration is a major health problem and its management involves multidisciplinary approach. This review aims to provide a synopsis of the current management strategies of diabetic foot ulcers, from prevention to the options for therapy. The authors believe that it may be useful to primary care physicians, nurses, podiatrists, dialectologists, and vascular surgeons, as well as all healthcare providers involved in the prevention or management of diabetic foot ulcers of skin integrity, providing a site vulnerable to microbial infection \(^12\). Peripheral arterial disease is 2–8 times more common in patients with diabetes, starting at an earlier age, progressing more rapidly, and usually being more severe than in the general population. It commonly affects the segments between the knee and the ankle. It has been proven an independent risk factor for cardiovascular disease as well as a predictor of the outcome of foot ulceration \(^13\). Even minor injuries, especially when complicated by infection, increase the demand for blood in the foot, and an inadequate blood supply may result in foot ulceration, potentially leading to limb amputation \(^14\). The majority of foot ulcers are of mixed etiology (neuro ischemic), particularly in older patients \(^15\). In patients with peripheral diabetic neuropathy, loss of sensation in the feet leads to repetitive minor injuries from internal (calluses, nails, foot deformities) or external causes (shoes, burns, foreign bodies) that are undetected at the time and may consequently lead to foot ulceration. This may be followed by infection of the ulcer, which may ultimately lead to foot amputation, especially in patients with peripheral arterial disease. Structural foot deformities and abnormalities, such as flatfoot, hallux valgus, claw toes, Charcot neuroarthropathy, and hammer foot, play an important role in the pathway of diabetic foot ulcers since they contribute to abnormal plantar pressures and therefore predispose to ulceration. Other risk factors for foot ulceration include a previous history of foot ulceration or amputation, visual impairment, diabetic nephropathy, poor glycemic control, and cigarette smoking. Some studies have shown that foot ulceration is more common in men with diabetes than in women \(^14,16\). Social factors, such as low socioeconomic status, poor access to healthcare services, and poor education are also proven to be related to more frequent foot ulceration \(^14,16\).

**Methodology**

Non-probability (purposive) sample application was conducted on a sample consisting of (50) patients having foot and leg ulceration who were selected from endocrine and diabetic center in Nasiriya city. An assessment format was constructed and designed depending on open format information and after reviewing the related literature, the objective of this assessment was to evaluate the need for the educational program. The assessment was conducted from january-2019 to July -2019 and the format was comp each respondent a period between 30-40 minutes. Researcher were fill out the format by himself. For those who were unable to ask to read and write, the researcher filled out the format herself with their original responses.

The data of present study were analyzed through the application of two statistical approaches, which may assist for the determination of the study results.

**Statistical Analysis**

A statistical questionnaire on diabetic foot disease was prepared and a sample of 50 patients was selected. The form consisted of two axes: The first on the socio-demographic aspect of the patient and the second on the clinical medical aspect.

The data from the form were classified and the variables were coded according to the requirements of the statistical program SPSS.

Since the variables used are qualitative and not quantitative, the correlation coefficient has been used to measure whether there are links between some variables and their analysis. The Spearman correlation coefficient is based approximately the vocabulary of each of the descriptive variables in question, with each given numeric value showing its order. Using these ranks, the correlation coefficient, called “grade”, can be calculated. The correlation coefficient can be calculated through the following formula:
\[
R_s = 1 - \frac{\sum D^2}{n(n^2 - 1)}
\]

Where \( n \) = number of observations

\( D \) = differences between two ranks of two observations

<table>
<thead>
<tr>
<th>Variables</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
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<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40-49 Year</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>50-59 Year</td>
<td>19</td>
<td>38</td>
</tr>
<tr>
<td>60-69 Year</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>70-80 year</td>
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<td>10</td>
</tr>
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<td><strong>Total</strong></td>
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</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>Male</td>
<td>34</td>
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</tr>
<tr>
<td>Female</td>
<td>16</td>
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<td><strong>Total</strong></td>
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<td><strong>Monthly income</strong></td>
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</tr>
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<tr>
<td>Enough to some extent</td>
<td>7</td>
<td>14.0</td>
</tr>
<tr>
<td>Not Enough</td>
<td>39</td>
<td>78.0</td>
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<td><strong>Total</strong></td>
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<td><strong>Residential area</strong></td>
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<td></td>
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<tr>
<td>Urban</td>
<td>33</td>
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</tr>
<tr>
<td>Rural</td>
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<td>34.0</td>
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<td><strong>Total</strong></td>
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<td>100.0</td>
</tr>
<tr>
<td><strong>Transport</strong></td>
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<td></td>
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<tr>
<td>Owns</td>
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<td>17.6</td>
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<td>Non owns</td>
<td>14</td>
<td>82.4</td>
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<td><strong>Total</strong></td>
<td>17</td>
<td>100.0</td>
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<tr>
<td><strong>housing</strong></td>
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<td>7</td>
<td>41.2</td>
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<td>Common property</td>
<td>6</td>
<td>35.3</td>
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<tr>
<td>Leasehold</td>
<td>3</td>
<td>17.6</td>
</tr>
<tr>
<td>Other</td>
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<td>5.9</td>
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<tr>
<td><strong>Total</strong></td>
<td>17</td>
<td>100.0</td>
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<td><strong>Marital status</strong></td>
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<td></td>
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<tr>
<td>Married</td>
<td>12</td>
<td>70.6</td>
</tr>
<tr>
<td>Unmarried</td>
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<td>11.8</td>
</tr>
<tr>
<td>Other (Divorce, widow)</td>
<td>3</td>
<td>17.6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>17</td>
<td>100.0</td>
</tr>
</tbody>
</table>
**Result**

Table (1) Distribution of the Study Sample by their General Information

Table (1) show that the percentage of age was 40% for age between 60-69 years, males was 68%, which is higher among the infected than the female rate of 32%, the patients residing in the city (urban) by 66%, the adequacy of the monthly income of the patient or not, 78% of them consider that their monthly income is not enough.

Table 1 Average age of the statistical sample of a diabetic foot and leg ulceration patients

<table>
<thead>
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<th>N</th>
<th>Valid</th>
<th>Missing</th>
<th></th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td></td>
<td>50</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
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<td>0</td>
</tr>
<tr>
<td>Std. Deviation</td>
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<td></td>
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<tr>
<td>Minimum</td>
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<td>40</td>
</tr>
<tr>
<td>Maximum</td>
<td></td>
<td></td>
<td>80</td>
</tr>
</tbody>
</table>

Table 2 showed that the average age of patients participating in the study was 59 years and that the ages of patients ranged between 40 and 80 years with 50% of patients between the ages of fifty -sixty years.

Table (3 ) Distribution of the Study Sample by their some variable

<table>
<thead>
<tr>
<th>Variables</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Self-Infected diagnosed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>17</td>
<td>34.0</td>
</tr>
<tr>
<td>yes</td>
<td>33</td>
<td>66.0</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100.0</td>
</tr>
<tr>
<td>Improvement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>17</td>
<td>34.0</td>
</tr>
<tr>
<td>yes</td>
<td>33</td>
<td>66.0</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100.0</td>
</tr>
<tr>
<td>Walking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>9</td>
<td>18.0</td>
</tr>
<tr>
<td>yes</td>
<td>41</td>
<td>82.0</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table (3) showed that the majority of patients discovered diabetes through their reviews, not the same thing. The proportion of patients diagnosed with the disease was 66% of the total sample. The percentage of patients who reported an improvement in their health through the therapy of carbothera therapy 66% indicated that the therapy had a positive effect on the patients, the form of the number of patients who responded to exercise daily, which is very useful to them, the number of patients who responded that they are walking Which were very useful to them, constituted a high rate of 82% of the patients in the sample.
Table (4) the relationship between the numbers of sessions of Carbothera therapy with improvement in the health status of the patient’s diabetic foot

<table>
<thead>
<tr>
<th>(1-5)</th>
<th>Num. of sessions</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(6-10)</td>
<td>(11-15)</td>
</tr>
<tr>
<td>Improvement</td>
<td>no</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>yes</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>16</td>
</tr>
</tbody>
</table>

Table (4) showed the majority of patients (23 patients and 46% of total patients) who responded to therapy were among the 1 to 15 session sessions supporting the patient’s response to therapy after attending only one and a half sessions (two sessions a week). The relationship between Spearman and (0.248) indicates that a significant mean relationship at an error level of 8% between the urban and rural environment variable with improved carbothera therapy indicates that therapy is more effective when the patient has a more urban than rural environment. The patient to the consequences of disease in the city be committed to therapy and get better results, While the relationship between the mean and moral level of error does not exceed 9 per cent between the variable and the presence of monthly salary of the patient due to lack of improvement in the therapy of carbothera, indicating that the therapy is more effective when the patient a monthly salary. This result is for the patient’s mental state and the state of safety provided by the continuous monthly salary.

The correlation is very weak and there is no daily walk to the patient with improved therapy of carbothera through the value of the link (Spearman) and the amount (0.007), may appear this way because the patient has difficulty walking because of the disease and declare that he walked but did not exceed the steps were not simple you remember.

**Discussion of the Results**

The study comprised 50 participants with confirmed diagnosis of diabetes mellitus. Were more males than females, age between 60-69 years that is (due to the customs and traditions that require men to accompany women during their visit to the hospital for therapy). The patients residing in the city (urban), Which indicates that the incidence of the disease may relate to the nature of nutrition in the city and the natural nutrition in the countryside, the adequacy of the monthly income of the patient or not, of them consider that their monthly income is not enough, which indicates that insufficient income leads to “malnutrition and the lack of a suitable environment for living”. Improvement in their health through the therapy of carbothera therapy indicated that the therapy had a positive effect on the patients, the form of the number of patients who responded to exercise daily, the number of patients who responded that they are walking Which were very useful to them, constituted a high rate of the patients in the sample, but the statistical results did not show a correlation with the improvement of their health condition due to their inability to walk already and the decline of a few daily plans, which makes it useless healthily. This agree with the study by Bakker et al. 2016 (International Working Group on Diabetic Foot Editorial Board. The development of global consensus guidelines on the management and prevention of the diabetic foot.

**Conclusion**

The management of diabetic foot and leg ulcers remains a major therapeutic challenge, which implies an urgent need to review strategies and therapy in order to achieve the goals and reduce the burden of care in an efficient and cost-effective way. Questions remain as to which types of intervention, technology, and dressing are suitable to promote improvement, and whether all therapies are necessary and cost-effective as adjunctive therapies. Diabetic foot ulcers should be carefully
evaluated and the gold-standard therapy should be strictly applied in order to prevent amputation. Further clinical studies are needed to support the existing evidence regarding the clinical benefit of new approaches for the therapy of diabetic ulcers, and these approaches should be used only as add-on therapies to the gold-standard wound care.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of Nursing, Al-Muthanna University, Iraq and all experiments were carried out in accordance with approved guidelines.

**References**


Outcome of Endoscopic Endonasal Repair of CSF Rhinorhea Using Fascia Lata Graft

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²Assistant Professor, Kufa university, college of medicine, ³ENT Specialist, Gazi AL-Hariri Hospital for Specialised surgeries, Medical City, Baghdad, Iraq,
⁴ENT Specialist, Gazi AL-Hariri Hospital for specialised surgeries, Medical City, Baghdad, Iraq

Abstract

Cerebrospinal fluid rhinorrhea occur when there is communication between skull base and the nasal cavity. Nowadays endoscopic endonasal repair of CSF rhinorrhea become the preferred surgical intervention.

Setting: ENT department at Ghazi AL Hariri Hospital for specialised surgeries, Medical City, Baghdad, Iraq.

Aim: To assess the effectiveness of fascia lata as sealing graft in the endoscopic endonasal repair of CSF rhinorrhea.

Patients and Method: Fifteen patients present to our department with possible clinical diagnosis of CSF rhinorrhea. The patients submitted to full history, examination including nasal endoscopy, radiological investigation (CT and MRI) of the nose and paranasal sinuses and skull base and CSF analysis. Operative technique was endoscopic endonasal repair using fascia lata graft. With monthly follow-up.

Results: Successful rate noted in 93% of the patients. No recurrence of meningitis attacks among those who reported meningitis on their presenting illness.

Conclusion: Endoscopic endonasal repair of CSF rhinorrhea has less operative and postoperative complication. Fascia lata graft offers a good choice for the repair.

Key words: CSF rhinorrhea, fascia lata graft and endoscopic endonasal repair.

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E-mail: drlaith2006@yahoo.com
Ghazi AL-Hariri for specialised surgeries Hospital, Medical City Complex, Baghdad, Iraq. Fifteen patients (eight males and seven females) were with a possible clinical diagnosis of CSF rhinorrhea. The duration of symptoms ranged from two months to twelve years. All patients had failed conservative treatment.

All patients underwent a thorough clinical history, endoscopic examination of nasal cavity, and the glucose concentration of the nasal discharge (CSF) was analyzed. All our patients underwent computed tomography (CT) and magnetic resonance imaging (MRI); CT scan was helpful in showing fracture site in post-traumatic CSF rhinorrhea while MRI was beneficial in detecting meningocele. Inclusion criteria was both the traumatic and non-traumatic CSF leak. Patients with skull base tumor were excluded from the study. Endoscopic examination where done using local decongestant and topical anesthesia using 0 degree rigid scope and the findings were difficult to localize the exact site of CSF leak, but the leak was noted coming from sphenoethmoidal recess or frontoethmoid area other patients with an obvious meningocele was noted specially in pediatric age group. Our surgical option was endoscopic endonasal repair of CSF rhinorrhea using tensor fascia lata as sealing graft. All surgeries done under general anesthesia with oral endotracheal intubation. Patients lie in slightly reverse trendelenberg position. In cases werethe defect in the cribriform area or from sphenoethmoidal area ,we laterialized the middle turbinate to get access to those areas ,sometimes we did compete ethmoidectomy to get access to the fovea ethmoidalis, In cases were a meningocele is present we follow it till reaching its neck and visualize the defect which coming from. The most critical step in the repair was to cauterize all the mucosa surrounding the defect and denuded the bone by using a bipolar suction diathermy to help get a good ground for the graft. A fatty tissue which obtained from the patient thigh plugged into the defect (underlay),after that an appropriately sized tensor fascia lata graft ( 30% larger than the defect due to later shrinkage of the graft ) is insinuated into the defect a few millimeters.then a gelfoam sponge was inserted to support the graft and a Merocil (Kennedy) pack was inserted and it was removed at third or fifth day; once the packs were removed; an endoscopic examination is done to remove any crustation and to get the first look after the surgery .The patients were instructed to minimize their daily activity like weight lifting, upstairs, sex, and avoid constipation by eating rich fiber diet. Patients were advised for monthly visit for endoscopic examination and assessment.

Results

Fifteen patients were with diagnosed of CSF rhinorrhea their age range from two years to fifty five years. Duration of symptoms range from two months to twelve years, eight patients were male and seven patients were female (figure 1); six patients experienced CSF leak from the left side while the other nine patients had the leak from the right side (figure 2).
Eight patients had history of head trauma (of those, three patients had previous nasal surgery) while the remaining seven patients had non traumatic CSF rhinorrhea (figure 3).

Figure (3) traumatic versus spontaneous CSF leak

Of those fifteen patients, five patients reported history of meningitis (ranging from one to several attacks during their illness); the other patients had no such history, figure (4).

Figure (4) meningitis rate
Eight patients were found to have a meningocele during the surgery; five meningoceles were originated from the cribriform plate, two meningoceles were originated from fovea ethmoidalis, while one patient had a sphenoidal meningocele, figures (5),(6).

Regarding CSF rhinorrhea without meningocele; the sites of the leaks were; three patients had leaks from the cribriform plate; three patients had leaks from fovea ethmoidalis and one patient had leak from frontal sinus.
Only one patient presented with recurrence of CSF rhinorrhea one month after surgery. Other patients did not report any rate of CSF rhinorrhea all over the follow up period. Patients with previous attacks of meningitis, they were free from it after the surgery.

**Discussion**

In our country, CSF rhinorrhea had been treated surgically by neurosurgeons using open approach (craniotomy) for many years with its highly morbidities and mortalities. In the last few years a great move done in our otolaryngology department Ghazi AL Hariri hospital to treat such cases with CSF rhinorrhea by endonasal endoscopic approach. In our study male with CSF rhinorrhea more common than female. Yong-Gang Kong et al (2013), found seventeen male and five female among 22 cases with CSF rhinorrhea. Thibaut Van Zele et al (2013), found five female and one male among six patients. Majority of our cases (53.3%) were traumatic, which is similar to most other study. S. Schmerber et al (2006), found six pateints had spontaneous leak and sixteen patietns had traumatic CSF leak. Muhammad Umar Farooq and Murtaza Ahsan Ansari (2011), also found the same rate. In our study all patients submitted MRI, Bernardo Cunha Araujo Filho et al (2005), Order MRI only for suspected cases of meningoencephaloceles. José Alberto Landeiro et al (2004), ordered MRI for all patients. We had five cases of meningitis, Bernardo Cunha Araujo Filho et al (2005), report one case among 44 cases. Yong-Gang Kong et al (2013), found four cases among twenty two patients, Mazhar Husain et al (2006), found three pateints had meningitis among nine patients. Antibiotics cover used for all our patients, Friedman JA et al (2001), adopted antibiotics for all patients. We have eight cases with meningocele among our patients. Yong-Gang Kong et al (2013), had two cases among 22 patients. In our study, majority of our cases had leak from lateral lamella of cribriform plate 53.33%. Bernardo Cunha Araujo Filho et al (2005), found the same. Silva et al (2006), found sphenoid bone involved more commonly than ethmoid bone. The success rate was of CSF closure in our study was 93.33%. Kirtane et al (2005), had success rate of 96.63%. Bernardo Cunha Araujo Filho et al (2005), 80% success rate. Schick et al (2001), report success rate of 80%.

**Conclusion**

Nowadays endoscopic endonasal repair of CSF rhinorrhea become the preferred option for the repair of CSF rhinorrhea with fascia lata graft offer a good choice for the repair in both traumatic and spontaneous CSF leaks with high successful rate.

Financial disclosure
There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Gazi AL-Hariri Hospital for specialised surgeries, Medical City, Baghdad, Iraq and all experiments were carried out in accordance with approved guidelines.

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7. Schmerber MD, Ch. Righini MD, JP Lavielle, MD. Endonasal Endoscopic Closure of Cerebrospinal Fluid Rhinorrhea, Departments of Ear, Nose, and Throat Surgery and 2Neurosurgery, Grenoble Hospital, Grenoble, France. Copyright C 2001 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New York, NY 10001, USA.


Genotype and Haplotype of HLA-Class II in Type I and Type II Diabetes Mellitus in Iraqi Patients

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Abstract

Background: Diabetes is one of the common complicated disease associated with multiple influenced factors, immunological serological and molecular factors play a great role in susceptibility and diagnosis of this disease. HLA genotyping were reported to have an influencing in both types of diabetes.

Aim: The current study was conducted to investigate the association of human leukocytes genotypes in Diabetes patients.

Method: Case-control study enrolled 225 blood samples collected from patient attended to the Marjan Teaching Hospital- Hilla and 25 apparently healthy from October 2018 to May 2019. Class II HLA genotyping was performing for 60 patients with diabetes mellitus and 25 healthy unrelated controls by means of the PCR-SSP method. The diagnosis of T1D and T1ID was set up according to American Diabetes Association criteria.

Results: both types of diabetes were significantly associated with HLA-DR3. Associations were also observed with HLA –DQA105:01, the appearance of these two alleles differs in both T1D and T1ID. Conclusion. Certain HLA class II alleles, haplotypes, and genotypes have related to diabetes mellitus so it can be dependent as a genetic marker for susceptibility of this disease in Iraq.

Keywords: Type II diabetic, Type I diabetes, HLA class II, genotype, haplotype.

Introduction

Diabetes mellitus (DM) is a group of metabolic disorders characterized by a chronic hyperglycemic condition resulting from the defects in insulin secretion, insulin action or both.¹ Type 1 and type 2 diabetes consider the two main types, with type 2 diabetes constituting for the majority (>85%) of the total diabetes mellitus prevalence, According to the latest estimates, there are 425 million people with diabetes in 2017 and this number is expected to rise in 2045 to 629 million.² The genetic loci involved in the rejection of foreign organs knew as the major histocompatibility complex (MHC), and the MHC encodes highly polymorphic cell surface molecules. The human MHC is representing as the HLA (Human Leukocyte Antigen) system because these antigens first identified and characterized using alloantibodies against leukocytes.³ The encoding of HLA-DQ proteins are belongs to HLA-DQ genes and expressed on α and β chain at cell surface.⁴ The DQ region of HLA include two gene clusters, DQA1 and DQB1.⁵ The polymorphism of HLA had serologically significant impact and the Polymorphism at the HLA-DQB1 locus used to be determined serologically and recognized the specificities DQ1, DQ2, DQ3 and DQ4.⁶ The use of DNA typing techniques has increased the number of alleles. The allelic sequence diversity is also predominantly present in exon 2 and, except for DQB1*0201 and DQB1*0202, all alleles can be discriminated by PCR-SSOP in this exon. A large number of studies have demonstrated that specific alleles at the DRB1, DQA1, and DQB1 loci are strongly associated with diabetes.⁷,⁸ However, allelic variation at these loci cannot account fully for the pattern of HLA haplotype sharing among affected sib pairs.⁹

Material and Method

Patients and controls

Two hundred twenty-five blood samples were
collected from clinically diagnosed diabetes patients who regularly admitted by medical committee specialized diabetic center of marjan hospital (Babylon) from October 2018 to May 2019, the age of patients between (2-80) years including both sex male (112) and female (113), in addition to (25) samples were taken from apparently healthy human were taken from Babylon province as control. The study was approved by the Research Ethics Review Boards of the University of Babylon. This work was done by self funding and it is a part of M.Sc project for the first author with the agreement of university .

All participants provided written informed consent. Case Ascertainment. Patients with diabetes were diagnosed by a physician on the basis of the following criteria: a fasting glycemia ≥ 1.26 g/dL, an unexplained weight loss, signs of hyperglycemia (polyuria, polydipsia, polyphagia, and asthenia). These criteria were defined according to the recommendations of the American Diabetes Association.10.

Blood sampling

For each individual enrolled in the study, 3 ml of venous blood was collected in EDTA-treated tubes for DNA extraction, which performed according to the protocols recommended by the manufacturer (Favorgen / Taiwan).

**HLA genotype analysis**

HLA –DQA105:01 and HLA-DR3 genotyping was performed with PCR-sequence-specific primers (PCR-SSP).11. In a 20 µl mixture of 2.5 µl DNA, 1.5 µl from each forward and reverse of the primer, 5 µl master mix and 9.5 µl nuclease free water. The DNA amplification for HLA-DQA1 is includes an initial denaturation of 2 min in 94°C, 32 cycles of amplification (every cycle consists of a denaturation of 30 s in 94°C, a hybridization of primers during 30 s in 63°C, and an extension of 30 s in 72°C), and a final extension of 10 min in72°C, 11. While the process of DNA amplification for HLA-DR3 is same as DQA1, (exception the hybridization temperature was 52°C), then the PCR products were separated in 1.5 % agarose electrophoresis system using ethidium bromide then visualized with the gel documentation, with 100 bp-ladder (Bioneer, Korea) and photographed. The sequences of primers used for the amplification of the genes are presented in table 1.

**Table 1: Sequences of the couples of primers used for the amplification of the genes.**

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequences 5-3</th>
<th>Size(bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA -DR3</td>
<td>5′CACGTTTCTTGGAGTAC3′ 5′CGTAGTTGTGTCGTGCAGTAGT3′</td>
<td>237 bp</td>
<td>(Fagbemi et al., 2017)</td>
</tr>
<tr>
<td>HLA-DQA1-05:01</td>
<td>5′ACGGTCCCTCCTGAGC3′ 5′AGTTGGAGCGTATCAGAC3′</td>
<td>186 bp</td>
<td></td>
</tr>
</tbody>
</table>

**Statistical Analysis**

All data were statistically analyzed according to software program version20 SPSS statistical software (version 17; SPSS, Inc., Chicago, IL, USA). The association between TID, TIID and each identified HLA-DR/DQ alleles, haplotypes, and genotypes was assessed using the odds ratio with its 95% confidence interval (OR, CI 95percentage)

**Results**

The present study reveals a noticeable variety with HLA haplotype among type I and type II diabetes. HLA alleles discriminated by PCR assay. The distribution of HLA-DQA1 show high appearance in both types, out of 30 samples 26 show positive result 86.66% in type I and out of 33 samples 26 show positive result 78.78% in type II finally, statistical analysis showed no significant differences comparing diabetes patient and control subjects table 2.
Table 2: Human leukocytes antigen (HLA) DQA1 alleles’ distribution in type I and II diabetes.

<table>
<thead>
<tr>
<th>Model</th>
<th>Sample No.</th>
<th>Patients +ve results</th>
<th>patients-ve results</th>
<th>Healthy +ve results</th>
<th>Healthy -ve results</th>
<th>OR (CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>Percent</td>
<td>No.</td>
<td>Percent</td>
<td>No.</td>
<td>Percent</td>
</tr>
<tr>
<td>Type I</td>
<td>30</td>
<td>26</td>
<td>86.66%</td>
<td>4</td>
<td>13.33%</td>
<td>21</td>
<td>84%</td>
</tr>
<tr>
<td>Type II</td>
<td>33</td>
<td>26</td>
<td>78.78%</td>
<td>7</td>
<td>21.21%</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>63</td>
<td>52</td>
<td>82.53%</td>
<td>11</td>
<td>17.46%</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1: Agarose gel electrophoresis image for HLA –DQA1 haplotype at 100 v for 40 min. and 70 v for 30 min. M, marker 100bp, L1, L2, L3, L4, L5, L6, L7, L8, L9, L10, L11 give positive results and L12 give negative result.

The distribution of HLA-DR3 in type I and II showed 14 positive result with percentage 46.66% for TID and 11 positive result (36.66%) for TIID whereas the control revealed 8 positive result (32%), finally, statistical analysis showed significant differences comparing diabetes patient and control subjects, table no 3 reveals high appearance of this haplotype HLA DR3 among TID than TIID.

Table 3: Human leukocytes antigen (HLA) DR3 alleles’ distribution in type I and II diabetes.

<table>
<thead>
<tr>
<th>Model</th>
<th>Sample No.</th>
<th>Patients +ve results</th>
<th>patients-ve results</th>
<th>Healthy +ve results</th>
<th>Healthy -ve results</th>
<th>OR (CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Type I</td>
<td>30</td>
<td>14</td>
<td>46.66%</td>
<td>16</td>
<td>53.33%</td>
<td>8</td>
<td>32%</td>
</tr>
<tr>
<td>Type II</td>
<td>30</td>
<td>11</td>
<td>36.66%</td>
<td>19</td>
<td>63.33%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>25</td>
<td>41.66%</td>
<td>35</td>
<td>58.33%</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

Diabetes mellitus is a combined metabolic disorder that includes several complications and the incidence of diabetes has been increasing worldwide therefore various are relentlessly working out the possible role of a vast number of genes associated with this disease\textsuperscript{12}. Human leukocytes antigen represented many proteins that encoded by HLA genes, therefore HLA-DQ genes expressed as heterodimers of alpha and beta chains at the cell surface\textsuperscript{13}.

The distribution of HLA-DQA1 in the present study show high appearance in both types, out of 30 samples 26 show positive result 86.66% in type I and out of 33 samples 26 show positive result 78.78% in type II finally, statistical analysis showed no significant differences comparing diabetes patient and control subjects table 2.

Although the high appearance of this HLA-DQA1 type in both types of diabetes TID and TIID, statistical analysis show no significant differences between patients and control, this led to conclude that this haplotype had no risky effect on this population sample of diabetes patients.

HLA allele and haplotype frequencies vary considerably across ethnic groups\textsuperscript{14}. Researchers pointed that HLA-DQA1 \(\square\) 05:01, HLA-DQA1 \(\square\) 03:01 play arole in diabetes susceptibility particularly TID\textsuperscript{15}.

The analyses of HLA disease associations in different ethnic populations, due to differences in allele frequency distributions and patterns of linkage disequilibrium, can allow important general inferences of disease risk associated with specific alleles and their combinations.

The distribution of HLA-DR3 in type I and II showed that type I diabetes revealed percentage 46.66% and type II revealed a percentage 36.66% whereas the control revealed percentage 32%. Statistical analysis showed significant differences comparing two types of diabetes patient.

On contrary of HLA DQA, HLA DR3 appear in less percentage in diabetes patients particularly in TIID, this may be due to the protective role of this haplotype in this population samples of diabetes. The increased risk of DR3/4-DQB1*0302 heterozygotes relative to DR3/3 and DR4/4 genotypes has led to the hypothesis that the trans-complementing DQ heterodimers are more effective in presenting diabetogenic epitopes to T-cells\textsuperscript{16}.

Type I diabetes has the strongest association with HLA-DQA1 and DR3 similar result were also reported by\textsuperscript{15}.

Studies on DRB1 and DQB1 allele distributions and the importance of genotype context support the genetic associations observed in previous studies\textsuperscript{17,18}.

Variable results on DQA1 and DR3 genotypes in...
this study observed the risky and protective role of these genotypes in TID, TIID and healthy individuals. This is compatible with the DQ heterodimer encoding by DQA1*0501 and DQB1*0302 explanation.19

**Conclusion**

This study was designed to assess the associations of HLA class II alleles, haplotypes, and genotypes with the risk of developing T1D and T2D in Iraq. Certain HLA class II alleles, haplotypes, and genotypes were related to diabetes and may be used as genetic susceptibility markers to diabetes. Further studies of HLA and diabetes mellitus in Iraq are needed to confirm the present results and to provide data for the development of screening assays and for better management of patients with diabetes at the onset of disease.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the college of Science for women, University of Babylon and all experiments were carried out in accordance with approved guidelines.

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Cloud Point Extraction, Preconcentration and Spectrophotometric Determination of Co (II) and Cu (II) using 15-Crown-5

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²College of Pharmacy, University of AL-Qadisiyah , Diwaniyah, Iraq

Abstract

In the current study, a cloud point extraction (CPE) with UV-vis spectrometry for detection of Cu (II) and Co (II) ions with 15-crown-5 as a chelating agent was developed to be more selective and sensitive. The CPE method is improving the analytical signal and permits the preconcentration of metals ions. After phase separation induced by change temperature to become higher more than the temperature of the cloud, the point is performed with centrifugation. The Co (II) and Cu (II) ions are determined in the enriched phase by UV-vis spectrometry. Several factors affecting the extraction efficiency were optimized, e.x. pH, the concentration of the chelating agent, non-ionic surfactant concentrations, and cloud point temperature. Under optimum conditions, a linear calibration graph in the range of 0.5-5 μg mL⁻¹ of Co (II) in the initial solution with r² = 0.9994 (n=8) and 0.2–7μg of Cu (II) in the initial solution (mL-1) with r² = 0.9991 (n=8) were obtained. Detection limits of 0.4and 0.1μg are for Co(II) and Cu(II) along with preconcentration factors (66 and 50) for these ions, respectively. The method was used for detection of Co(II) and Cu(II) in some food samples.

Keywords: Cloud point extraction, preconcentration, spectrophotometric

Introduction

CPE Cloud Point Extraction method which is interest with the separation method metal ions field by forming small volume of the surfactant-rich phase (colloidal system) as a result of heating non-ionic aqueous solution of surfactant to a certain temperature at which the solution become turbid ,then the colloidal solution is separated into a small volume of surfactant-rich phase and an aqueous phase(1-6). A list of advantages of this method other than analytical methods because of i-it green method to avoid hazardous solvents ii- its selectivity for obtaining high coefficient of preconcentration iii-its economic for capital cost iv- its rapidity for reduce the time(7-9) iv-simplicity application for separation and purification of a variety samples of pharmaceutical products, vital and solid,and enviromental(10-16).

A previous study used CPE methodology coupled with flame atomic absorption,(17-22) high performance liquid chromatography,(23) Ultrasonically modified Amented,(24-25) Ultrasound,(26-27) atomic absorption spectrometry,(28)Spectrophotometric technique(29) and total reflaction X-ray fluorescence(30).

Supramolecular chemistry are strong linked to molecular recognition chemistry ,which investigates now host molecules that do the recognizing quests molecules that are recognized although molecular recognition chemistry is sometimes called host-guest chemistry ,the main concept associated with molecular recognition is the “Lock and Key “concept proposed by Email Fisher at the end of the nineteenth century 31. According to the structures and sizes of various crown ethers are forming stable and selective complexes with metal ions including alkali and alkali earth metal catoin although ability of these macro cyclic ligands as electron pair molecules towards neutral molecular iodine and with δ- or π- acceptors(35-37). In this present work we report the formation of complex 15Crown5 with copper and cobalt in ethanol solution. This work focused on the suitability of CPE combination with UV-Visible spectrophotometer.
The experimental

The apparatus

Spectrophotometer (A Shimadzu UV–vis 2101) was used for making experiment studies. Determinations of pH were measured using a (Wellhem, Model 7110) (Germany) digital pH meter. The water bath is used for studying temperature (OPTIMA co, made in Japan). A centrifuge (Hettich co., made in Germany) was used to the separation.

Reagents and Solutions

Doubly Distill water was used in the study. The nitrate salts of cobalt and copper (Merck) solution of 1000 μg/mL were prepared by dissolving the appropriate amount of nitrate salt. The solutions were produced by dilution by doubly distil water. The nonionic surfactant Triton X-114 was provided (Acros Organics company, New Jersey, made in the USA).

Suggested methods for Cloud point extraction

For making cloud point extraction, it requires 10 ml aliquot solution containing 0.5–5 μg mL⁻¹ of Co(II), 0.2–7 μg mL⁻¹ of Cu(II). Then, a solution containing 15C5 dissolved in Triton X-114 was added to the aliquoted solution at a suitable pH. The phase separation occurs when heated the solution in the water bath at 60 °C for 15 min, So accelerating phase separation by using centrifuging the solution at 5000 rpm for 10 min. Then cooling, the surfactant-rich phase changed to viscous, and the aqueous phase was easily removed using a syringe pipette. The surfactant-rich phase was made up to 1.0 mL by adding ethanol and Trans to a quartz cell to the determination of the absorbance at 290,252 Nanometer.

Application of foods samples:

Heating spinach sample (40) gram for three hours in silica crucible on heater and the charred substances was moved to oven for heating 24 hours at 650°C. then cooled, after that treated by nitric acid (10) mL and H₂O₂ (3) mL at concentration (30) and return to the oven for two hours at 650 °C, and shouldn’t any organic compound. Treatment of final residue by nitric acid (3) mL and convert by evaporated to the fumes. Final solid was kept in distal water, then keep at pH (4) made up to 25 mL. Black tea one gram, green tea one gram and tomato sauce one and a half gram were digested with the mixture of 8 mL concentrated nitric acid, H₂O₂ (2) mL at a concentration (30%) in microwave and dilution it by deionized water (50) mL. Also, the blank digest too submitted for the same way. The CPE methods were used to the final solutions. The analyte ions levels were determined by the procedure provided in section 2.3.

The Results and the Discussion

Absorption spectra and Characteristics of the method:

The absorption spectra of Co (II)-15-C-5 and Cu (II)-15-C-5 complexes in the surfactant-rich phase after CPE procedure against reagent blank are shown in Fig. 1 the detection wavelengths were chosen to be 290 and 252 nm. The stoichiometry of 15-Crown-5 complexes with Co (II) and Cu (II) was examined by mole ratio method, and the continuous variation was found to be 1:1. The preconcentration factor results in by dividing aqueous phase volume to the preconcentrated phase volume. Table 1 summarizes the analytical characteristics of the method under the optimum experimental conditions.

![Fig. 1: Absorption spectra (a) Co(II)-15-C-5 complex, Co (II) = 2.5 μg mL⁻¹, 15-C-5 = 1 x 10⁻² M , pH = 4 , 0.4 mL of 2 % (v/v) Triton X-114 (b) Cu(II)-15-C-5 complex, Cu (II) = 2.5 μg mL⁻¹, 15-C-5 = 9 x 10⁻³M , pH = 4 , 0.5 mL of 2 % (v/v) Triton X-114.](image-url)
Table (1): showed parameter methods that used for detection values of cobalt and copper by using CPE.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Co (II)</th>
<th>Cu (II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{\text{max}} ) (nm)</td>
<td>290</td>
<td>252</td>
</tr>
<tr>
<td>Regression equation</td>
<td>[ y = 0.1981 x + 0.0006 ]</td>
<td>[ y = 0.2886 x + 0.0102 ]</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9993</td>
<td>0.9993</td>
</tr>
<tr>
<td>C.L. for the slope (b±tσb) at 95%</td>
<td>0.1981±0.0176</td>
<td>0.2886±0.0011</td>
</tr>
<tr>
<td>C.L. for the intercept (a±tσb) at 95%</td>
<td>0.0006±0.0491</td>
<td>0.0102±0.0303</td>
</tr>
<tr>
<td>Concentration range (µg mL(^{-1}))</td>
<td>0.5–5</td>
<td>0.2–7</td>
</tr>
<tr>
<td>Limit of Detection (µg mL(^{-3}))</td>
<td>0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>Limit of Quantitation (µg mL(^{-1}))</td>
<td>1.56</td>
<td>0.65</td>
</tr>
<tr>
<td>Sandell's sensitivity (µg cm(^{-2}))</td>
<td>1.0×10(^{-3})</td>
<td>6.257×10(^{-3})</td>
</tr>
<tr>
<td>Molar absorptivity (L mol(^{-1}) cm(^{-1}))</td>
<td>5.7726×10(^{4})</td>
<td>1.0161×10(^{4})</td>
</tr>
<tr>
<td>Composition of complex (M: L(^*))</td>
<td>1:1</td>
<td>1:1</td>
</tr>
<tr>
<td>RSD% (n=7) at 3 µg mL(^{-1})</td>
<td>3.3%</td>
<td>2.8%</td>
</tr>
<tr>
<td>Preconcentration factor</td>
<td>66.66</td>
<td>50</td>
</tr>
</tbody>
</table>

Optimizing of CPE parameters:

**Effect of pH:**

The separation of ions includes primary production sufficient hydrophobic with metal-chelate to be extraction of a little amount of surfactant-rich phase \(^{(38)}\). pH test is the main parameters \(^{(39)}\). PH effect to the CPE method was applied in (1–5) range. Adjusting of PH values is with \(\text{HNO}_3\). Figure (2) showed a pH effect on the percentage of extraction; it included the extraction percentage increasing with nitric acid decreasing. The final results, pH (4) were chosen.

![Fig. 2: PH effect on the determination of Cu (II) and Co (II)](image)

Conditions: Co (II) = 2.5 µg mL\(^{-1}\), 15-C-5 = 1×10\(^{-2}\) M, 0.4 mL of 2 % (v/v) Triton X-114 (b) Cu(II)-15-C-5 complex, Cu (II) = 2.5 µg mL\(^{-1}\), 15-C-5 = 9×10\(^{-3}\) M, 0.5 mL of 2 % (v/v) Triton X-114.
Effect of 15-Crown-5 concentration:

The effect of 15-Crown-5 on the extraction and determination of Co (II) and Cu (II) complexes were investigated in the range $2.0 \times 10^{-3}$ to $2.0 \times 10^{-4}$ mol L$^{-1}$, the results present in Fig. 3. The extraction will result in high concentrated of 15-Crown-5 and remained almost constant. That explains that micelles are filled by the chelating agent. At higher concentration, extraction efficiency was decreased. That due to the free 15-Crown-5 competes with the complexes in extraction to surfactant-rich phase results in low extraction$^{(40,41)}$. Therefore, a concentration of $1.0 \times 10^{-2}$ and $9.0 \times 10^{-3}$ mol L$^{-1}$ of 15-Crown-5 was selected as the optimum concentration Co (II) and Cu (II).

Triton X-114 Effect:

One of a critical factor that in the CPE is the concentration of surfactant, Triton X-114 is non-ionic surfactant wide using in CPE due to its achieves a good cloud point extraction that means increase the extraction efficiency by reducing the phase volume ratio, results in improvement of concentration factor. Where Triton X-114 present in markets at high purity, with low point temperature, low cost and toxicity and the surfactant-rich phase (high density)$^{(42,43)}$. The preconcentration efficiency depends on the Triton X-114 level (0.001-0.015) as range M, as shown in Fig. 3. At lower concentrations of the surfactant, The highest extraction efficiency for cobalt and copper ions was obtained with 0.007 and 0.009 M Triton X-114. The extraction decreases with increasing of the surfactant amounts. That may be belonging to increasing in viscosity and volume of the micellar phase leading to poor sensitivity$^{(44)}$. An amount of 0.4 and Triton X-114 (0.5 Ml) were selected for subsequent experiments.

Fig. 4: Effect of Triton X-114 concentration on the determination of Co (II) and Cu (II) Conditions: Co (II) = 2.5 μg mL$^{-1}$, 15-C-5 = 1 x 10$^{-2}$ M, pH = 4, Cu(II)-15-C-5 complex, Cu (II) = 2.5 μg mL$^{-1}$, 15-C-5 = 9 x 10$^{-3}$ M, pH = 4.
Effect of Time and Temperature of Equilibration Incubation:

Determination of incubation is done by temperature and time. As well as, the extraction process depends on temperature. Figure (5) showed the influence time and temp of the incubation of Cobalt and copper. The cloud point temperature of Triton X-114 is (23-25) °C in (0.1–5) weight percentage, heating more than ten °C is important for getting good separation (45). When temperature or incubation time is an increase, it will increase Co (II) and Cu (II) extracted amount in the surfactant-rich phase with quantitative separation after fifteen minutes in 60 °C (water bath). The prolonged times is necessary to apply it at high temperatures for satisfactory mange extraction (46).

Wide ranges are used in our study (30–70) °C as temperature degree and (5–30) minutes as a time. Time (15) minutes and temperature (45) °C were optimum conditions for extraction.

![Fig. 4: Effect of Triton X-114 concentration on the determination of Co (II) and Cu (II) Conditions: Co (II) = 2.5 µg mL⁻¹, 15-C-5 = 1 x 10⁻² M, pH = 4, 0.4 mL of 2 % (v/v) Triton X-114, Cu(II)-15-C-5 complex, Cu (II) = 2.5 µg mL⁻¹, 15-C-5 = 9 x 10⁻³ M, pH = 4, 0.5 mL of 2 % (v/v) Triton X-114.](image)

The effect of centrifuge conditions:

Centrifugation was accomplished for separating the aqueous and surfactant-rich phase in the suggested procedure. (5000) recycled per minute and ten minutes are best phase separation (47,48).

Effect of Foreign Ions:

The interferences are studied in the preconcentration stage, 15-crown-5 react cations or analytes may react with species and the extraction efficiency become low. The solution (10) ml consist of (3) µg mL⁻¹ Co (II) and Cu(II) as Table (2).

<table>
<thead>
<tr>
<th>Ion</th>
<th>Added as</th>
<th>Tolerance Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>K⁺ , Na⁺</td>
<td>KCl, NaCl</td>
<td>1000</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>MgCl</td>
<td>1000</td>
</tr>
<tr>
<td>Pr³⁺</td>
<td>Pr(NO₃)₃</td>
<td>500</td>
</tr>
<tr>
<td>La⁺</td>
<td>La₂O₃</td>
<td>1000</td>
</tr>
<tr>
<td>I⁻</td>
<td>Resublime</td>
<td>1000</td>
</tr>
<tr>
<td>Li⁺</td>
<td>d iodine</td>
<td>1000</td>
</tr>
<tr>
<td>Ag⁺</td>
<td>LiCl, AgNO₃</td>
<td>750</td>
</tr>
</tbody>
</table>

Table (2): Effects of the matrix ions on the recoveries of the examined metal ions (N=3)
Applications and Accuracy:

The suggested procedure was used to determination of the Co(II) and Cu(II) according to Section 2.4 in Spinach, Tomato Sauce, Green tea and Black tea provided from local markets. The results are shown in Tables 1. The values measured by at ranged (96-101) %, the results indicate that confirm the accuracy of the procedure.

Table (3): Contents of analyte ions in the food sample. (N = 3).

<table>
<thead>
<tr>
<th>Food</th>
<th>Co</th>
<th>Cu</th>
<th>R.S.D</th>
<th>Recovered (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spinach</td>
<td></td>
<td></td>
<td>98.8</td>
<td></td>
</tr>
<tr>
<td>Co</td>
<td>0</td>
<td>8.61</td>
<td>2.3</td>
<td>98.8</td>
</tr>
<tr>
<td>Cu</td>
<td>2.5</td>
<td>11.08</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>14.52</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>16.92</td>
<td>2.1</td>
<td>96</td>
</tr>
<tr>
<td>Tomato Sauce</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co</td>
<td>0</td>
<td>0.82</td>
<td>3.4</td>
<td>100.4</td>
</tr>
<tr>
<td>Cu</td>
<td>2.5</td>
<td>3.33</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1.50</td>
<td>4.1</td>
<td>97.6</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>3.94</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>Black tea</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co</td>
<td>0</td>
<td>1.93</td>
<td>3.3</td>
<td>101.2</td>
</tr>
<tr>
<td>Cu</td>
<td>2.5</td>
<td>4.46</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>2.41</td>
<td>3.1</td>
<td>99.2</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>4.89</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>Green tea</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co</td>
<td>0</td>
<td>1.30</td>
<td>2.1</td>
<td>97.2</td>
</tr>
<tr>
<td>Cu</td>
<td>2.5</td>
<td>3.73</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>7.99</td>
<td>1.8</td>
<td>96.4</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>10.40</td>
<td>0.89</td>
<td></td>
</tr>
</tbody>
</table>

A comparison between previously studies and the suggested method of pre-concentration and Cu(II) and Co(II) determination as (Table 4) which indicates the method R.S.D. and lower detection limit and the methods are the procedure for detection small Co(II) and Cu(II) in food by use spectrophotometer.
Table 4: Comparison between merit methods with previous studies

<table>
<thead>
<tr>
<th>Sample</th>
<th>reagent</th>
<th>surfactant</th>
<th>LOD (ng mL⁻¹ PF detection)</th>
<th>PF</th>
<th>detection</th>
<th>.Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>water and food samples</td>
<td>PTSC-1</td>
<td>Triton X-114</td>
<td>Co 1, Cu 0.6</td>
<td>25</td>
<td>25</td>
<td>49</td>
</tr>
<tr>
<td>Environmental samples</td>
<td>MPKO</td>
<td>Triton X-114</td>
<td>Co 0.1, Cu 0.1</td>
<td>67</td>
<td>65</td>
<td>50</td>
</tr>
<tr>
<td>tap water, snow water</td>
<td>PAN</td>
<td>Triton X-114</td>
<td>Co 0.2, Cu 0.2</td>
<td>25</td>
<td>25</td>
<td>51</td>
</tr>
</tbody>
</table>

**Conclusion**

Extraction of ions of cobalt and copper is done with 15-crown-5 in non-ionic surfactant Triton X-114 phase. Extraction of complete elements was done by 15-crown-5 because of the hydrophobic production. The molar ratio is determined. Simplicity, Ecological safety, metrological characteristics, the sensitivity and convenience of the procedures used for extraction of the organic compounds. The results found these methods could use it for preconcentration and separation of cobalt and copper ions.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Department of Chemistry, College of Education, University of AL-Qadisiyah, Diwaniyah, Iraq and all experiments were carried out in accordance with approved guidelines.

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Hormonal and Immunological Study in Serum of Pregnant albino rats Treated with Nickel Oxide Nanoparticles

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Abstract
The present study was aimed to elucidate the effect of Nickel oxide nanoparticle (100mg/kg b.wt) on some parameters in pregnant Albino rats which including serum reproductive hormonal level, Leutinizing Hormone (LH), Follicle stimulating Hormone (FSH) and Testosterone (Test), and some Immunoglobulin levels (IgA, IgG, IgM) for different period of pregnancy (12 and 14 days). The hormonal results showed significant (P≤0.05) increased of treatment groups as compared with control groups. Immunological results showed significant decreases in treated groups as compared with control groups. It could be concluded that increasing concentrations of NIO-NPs and durations of exposure leads to negative effects on the pregnant albino rat.

Key words: NIO, nanoparticles, albino rats, hormones, immunoglobulin.

Introduction
Nickel is a silver-white metallic chemical element that is naturally present in the Earth’s crust. Because of its unique physical and chemical properties, being tough, harder than iron, ferromagnetic, having good plasticity and highly resistant to rusting and corrosion, nickel and its compounds are widely used in industry. Nickel is an essential element for at least several animal species. These animal studies associate nickel deprivation with depressed growth, reduced reproductive rates, and alterations of serum lipids and glucose. Nickel is known as a potentially harmful element for humans. Its concentration in the environment can rise due to industrial activities. Human exposure to nickel or its compounds has the potential to produce a variety of pathological effects, which may include cutaneous inflammations such as swelling, reddening, eczema and itching on skins, and may also include allergy reactions and teratogenicity in the human body. Nickel is capable of evoking dual responses in the human immune system. Experiments conducted in humans and in rodents have shown that nickel exhibits both immune modulatory and immune toxic effects. Number of immunological and lymphoreticular effects have been reported in humans and animals exposed to nickel. In 38 production workers exposed to nickel (compound not specified), significant increases in levels of immunoglobulin G (IgG), IgA, and IgM and a significant decrease in IgE levels were observed. Significant increases in other serum proteins, which may be involved in cell-mediated immunity (including α1-antitrypsin, α2-macroglobulin, ceruloplasmin) were also noticed. Nickel and chromium significantly depressed the circulating antibody response of rats immunized with a viral antigen, with the greatest decrease in antibody titers noted in animals receiving the metal two weeks before the initial antigen dose. Several studies have examined the relationship between nickel exposures and acquired immune function. Exogenous chemicals can interfere with the normal functioning of the HPG axis, resulting in reduced fertility or even infertility in both females and males. Ni NPs effect the serum sex hormone levels (FSH, LH, E2 or T) in female and male rats. Some study demonstrate that Ni NPs increased significantly the level of serum FSH and LH, and decreased E2 this effect associated with dose-dependent in females. The results indicate the effects of Ni NPs on the female rat ovarian reserve. It is probably an indication of the decreased level of serum E2 and ovarian hormone secretion following ovarian damage with Ni NPs, which increased the level of serum FSH.

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and LH by negative feedback. Meanwhile, the male rat serum FSH, LH and T content analysis showed the levels of FSH and T were decreased significantly by Ni NPs treatment.

Materials and Method

Animals

Animals with weight of 195-280 gm and aged of 2.5-3 months were obtained from the animal house of the Biology Department/College of Science at University of Babylon. Animals were put inside special cages for breeding with length of 25 cm, 18 cm width and 19.5 cm height and stayed about 30 days. The cages were covered with sawdust, which replaced three times weekly with the care of hygiene and sterilization. The animals were provided with food and water ad libitum. The animals were housed in special rooms with controlled conditions of temperature (24±10°C) and natural light periods (12 hours light/dark). Each two females were put with one male (for mating) in special plastic cages and strung metal caps with dimensions of 40 cm length, 25 cm width and 19.5 cm height. After ensuring the pregnancy by observing vaginal plug and vaginal smear, this day regarded as 0th day of gestations (GD=0). Pregnant rats divided in five groups (n=4). Each two females were put with one male (for mating) in special plastic cages and strung metal caps with dimensions of 40 cm length, 25 cm width and 19.5 cm height.

Nanoparticles Dose Preparation

Determination of Nickel oxide doses were depended on the animals body weight. 25-PPM doses of nanoparticle suspension was prepared and mixed with distilled water.

Animals Anesthesia

The pregnant rats were anesthetized with chloroform in 12 and 14 days of pregnancy. Pregnant rats were put after anesthesia on dissection bowl, the fore and hind limbs were fixed by fine pins, the pregnant’s abdomen was opened by a sharp scissors and take blood sample by heart puncture.

Blood collection: Blood samples were collected via the left ventricular cardiac puncture into sterilized EDTA tubes and gel tube to separate the serum quickly and then centrifuged at 3000 rps for 5 minutes. The serum samples were stored frozen until used.

Hormonal Assay: Serum Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH) and Estradiol were measured by using ELISA (Monobind Company, USA) for both control and treatment animals.

Results

Effect of Nickel Oxide nanoparticles on Luteinizing Hormone in Pregnant Albino Rats for 12, 14 Days of Pregnancy.

The result of the present study showed that the LH hormone means increases significantly (P≤0.05) in treated group (12, 14 days) with NIO (100 mg/kg b.wt) (1.1 ±0.010, 1.28 ± 0.043) (pg/ml) respectively, as compared with control group (0.8263 ± 0.0251, 0.836 ± 0.0251) for 12, 14 days respectively (Figure-1).

Effect of Nickel Oxide Nanoparticles on Follicle Stimulating Hormones in Pregnant Albino Rats for 12 and 14 Days of Pregnancy.

FSH means in present study increases significantly (P≤0.05) in treated group with NIO (100 mg/kg b.wt ) (1.023 ±0.032, 1.16 ± 0.051) (pg/ml) as compared with control group (0.8 ± 0.004, 0.8 ± 0.003 pg/ml) 12 and 14 days respectively (Fig.2).
Effect of Nickel Oxide nanoparticles on Testosterone Hormone in Pregnant Albino Rats for 12 and 14 Days of Pregnancy.

The present study showed significant increases (P≤0.05) of Testosterone in treated group with NIO (100 mg / kg b.wt ) (0.61 ±0.001 , 0.63 ±0.001 pg/ml), as compared with control group (0.541 ±0.013 , 0.52 ±0.08 pg/ml) for 12 and 14 days respectively (Figure-3).

Effect of Nickel Oxide nanoparticles on Immunoglobulin A in Pregnant Albino Rats for 12 and 14 Days of Pregnancy.

The result in the (Figure-4) revealed that the IgA, showed significant (P≤0.05) decreases in the treated group with NIO (100 mg / kg b.wt) (16.29 ± 0.292, 14.3± 0.22 pg/ml) as compared with control group (19 ± 0.386, 18.63± 0.52 pg/ml) for 12 and 14 days respectively.

Effect of Nickel Oxide Nanoparticles on Immunoglobulin G in Pregnant Albino Rats for 12 and 14 Days of Pregnancy.

The result in the (Figure-5) revealed that the IgG, showed decreases significant (P≤0.05) in the treated group with NIO (100 mg / kg b.wt) (95 ± 1 , 82.66± 1.52 pg/ml) respectively, as compared with control group (119 ± 1 , 118.3± 1.52 pg/ml) for 12 and 14 days respectively.

Effect of Nickel Oxide Nanoparticles on Immunoglobulin M in Pregnant Albino Rats for 12 and 14 Days of Pregnancy.

The result in the (Figure-6) revealed that the IgM showed significant (P≤0.05) decreases in the treated group with NIO (100 mg / kg b.wt ) ( 16.35 ± 0.19 , 14.46± 0.21 pg/ml) respectively, as compared with control group (19.62 ± 0.06 ,18.24± 0.02 pg/ml) for 12 and 14 days respectively.

Discussion

Effect of Nickel Oxide Nanoparticles on Luteinizing, Follicle stimulating and Testosterone Hormones in Pregnant Albino Rats for 12 and 14 days of pregnancy.

The present study showed significant (P≤0.05) increases in treated group with NIO (100mg /kg b.wt) as compared with control group for 12 and 14 days of pregnancy this change of hormones could be resulted
from the hypothalamic–pituitary–gonadal (HPG) axis is the hormone system whereby the hypothalamus secretes so-called releasing hormones, which are transported via the blood to the pituitary gland. There, the releasing hormones induce the production and secretion of gonadotropins (i.e., LH and FSH), which in turn are transported by the blood to the gonads (i.e., the ovaries and testes).

Generally speaking, in females, LH and FSH stimulate the ovarian follicle that contains the maturing egg to produce estradiol. After ovulation has occurred, LH also promotes production of progesterone. Both hormones participate in a negative feedback mechanism through most of the menstrual cycle, suppressing GnRH release from the hypothalamus and LH release from the pituitary (11).

Exogenous chemicals can interfere with the normal functioning of the HPG axis, resulting in reduced fertility or even infertility in both females and males, the effects of Ni NPs on aspects of serum sex hormone levels (i.e., FSH, LH, E2 or T) in female and male rats. Increases of Ni NPs level in serum FSH and LH, with significant and dose-dependent in females. It is probably an indication of the decreased level of serum E2 and ovarian hormone secretion following ovarian damage with Ni NPs, which increased the level of serum FSH and LH by negative feedback 12.

The change of hormone reproductive levels indicates the abnormal reproductive axis function, which correlated with male and female infertility 13. However, the mechanisms by which NPs alter the functions of HPOA ultimately resulting in female infertility have not been investigated thoroughly 15. Meanwhile, the amount of researches about NPs having negative effects on HPOA is relatively small. It is certain that NPs with size of 36 nm were significantly accumulated in cerebrum and cerebellum translocation via the olfactory nerve and increased with the exposure time 16. The sizes of NPs less than 90nm could disturb the balance of GnRH, FSH and LH, such as Ni, PEG-b-PLA(17) 18.

Other study showed that gold nanoparticles reduced the level of testosterone An- other assumption is that the nanoparticles could affect the gene expression of the protein that is involved in the transport of cholesterol into the inner membrane of mitochondria, and eventually inhibit the conversion of cholesterol to pregnenolone and reduced the level of testosterone. Karpenko (2013) studied toxic effects of cerium oxide nanoparticles on sex hormones and concluded that nanoparticle reduced glandular and testosterone secretion, the gold nanoparticles reduced the level of testosterone 19.

Effect of Nickel Oxide Nanoparticles on Immunoglobulin A, G and M in Pregnant Albino Rats for 12 and 14 Days of Pregnancy.

The results of the current study as in the table () revealed that the IgA, IgG and IgM, showed significant (P≤0.05) decreases in the treated group of (12, 14) days with NIO (100mg/kg b.wt), as compared with control group. The decreases of Immunoglobulins levels in pregnant females due to that the nanoparticles can also suppress the immune system which can weaken immune response against infections and cancerous cells. These immunosuppressive properties, on the other hand, can make nanoparticles useful in preventing transplant rejection, in treating inflammatory and autoimmune diseases, and in delivering immunosuppressive drugs 20.

Nanoparticle properties are responsible for immunosuppressive effects. While some nanoparticles are used to deliver immunosuppressive drugs, others have their own immunosuppressive properties. Shen et al. 22 have shown that Fe3O4 nanoparticles weaken the antigen-specific humoral response and T cell cytokine expression in ovalbumin-challenged mice. Mitchell et al. 23,24 reported that multi-walled carbon nanotubes (MWCNTs) suppressed systemic humoral immunity in mice. Some nanoparticles have been shown to possess anti-inflammatory properties. CeO2 nanoparticles were reported to reduce ROS and the level of inflammatory cytokines IL-6 and TNF-α in murine macrophages 25. After the immunization of mice with a C60 fullerene derivate conjugated to bovine thyroglobulin, they produced IgG antibodies specific to fullerenes. Other researchers were not able to detect fullerene-specific antibodies, even when they used a carrier molecule 25.

Conclusion

The hormonal results showed significant (P≤0.05) increased of treatment groups as compared with control groups. Immunological results showed significant decreases in treated groups as compared with
control groups, It could be concluded that increasing concentrations of NIO- NPs and durations of exposure leads to negative effects on the pregnant albino rat.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Babylon University/College of Science, Iraq and all experiments were carried out in accordance with approved guidelines.

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Assessment of Mental Health of Healthcare Professionals Working in the Infectious Diseases Units: Comparative Study

Maysaa Niama Aboalshon¹, Hayder H. AL-Hadrawi², Ammar Abbas Shalan²

¹ MScN, ² PhD, College Nursing, University of Babylon

Abstract

Background: Health care professionals working in infectious diseases units or hospitals are at higher risk for being affected by certain contagious pathogens than other professional groups through direct contact, equipment, and contaminated supplies; therefore, they are working under ongoing stress that negatively impacting their psychological well-being.

Aims: This study aims to assess the mental well-being of healthcare professionals working in infectious diseases units and compare these results to the score of other healthcare professionals working in other units.

Methodology: A comparative study design using a convenience sample of (N=300) healthcare professionals (150 working in the infectious diseases units and 150 working in different units). The General Health Questionnaire-28 was used to assess the mental health of study subjects.

Results: Recent findings indicate a statistically difference between study group and comparative group (p= <0.05), which means that healthcare providers working in the infectious disease units are at higher risk for psychological disturbance than the comparative group. Moreover, other work factors are found to have significant relationship with professionals’ mental health, such as infection prevention trainings, years of experience in infectious diseases units, availability of personal protection equipment and hand hygiene, and number of patients in the unit.

Conclusion: Working in high risk work environment increases the concern about personal safety and impose ongoing stress, which negatively influence professional’s mental well-being. More training about infection prevention and provide personal protection materials help reducing the risk of disease transmission among patients and to health care providers.

Keywords: Emotional Intelligence, Work-related Stress, Psychiatric Nurses

Introduction

Most healthcare professionals are vulnerable to the risks of workplace; especially those who expose to blood, body fluids, and injuries that could be contaminated with infectious microorganisms (15,20). Death rates are more likely to be higher among healthcare professionals (especially nurses and doctors) who work in infectious diseases units, as a result of their role as frontline professionals in caring for clients with different infectious diseases 14. Health care professionals working in infectious diseases units or hospitals are at higher risk for being affected by certain contagious pathogens through direct contact, equipment, or contaminated supplies; consequently, they are working under an ongoing stress that affects their psychological health. Working in such units increases the demand on health professionals to increase the quality of hospital care, controlling infection, and protecting themselves at the same time, which impose additional stress and impact the professionals’ general health outcomes 4. The purpose of study is to assess the mental health outcomes of healthcare professionals working in the infectious diseases units and measure the difference in the mental health outcomes among healthcare professional working in the infectious diseases units and other units. Mild stress is consider essential to promote professionals’ productivity and responses; however, when the stress level is high, it negatively impact the health of individuals. Workplace challenges do not
often reported to psychological complaints; however, psychological health of professionals is impacted when they are unable to adapt effectively with these challenges. Stress is described an imbalance among the perceived needs, understand resources, and ability to cope with those needs, which can cause physical and psychological distress. Stigma is not only affecting clients with infectious diseases, it is another issue that faces healthcare professional who care for those clients as a result of the possibility of infection transmission to healthcare care employees. Consequently, it is also considered one of the main causes of perceived stress, depression, and anxiety. Professional mental health is directly and indirectly influenced by the stigma associated with infectious diseases through stress. The Importance of risk awareness and patients safety have been indicated in several research. According to, “nurses are expected to maintain a high level of awareness towards an infectious disease outbreak and ensure the safety of the public.” This critical role highlights the reason behind the high level of stress among nurses who work in communicable diseases units. Identifying the risk factors that cause professional stress among health professionals is a crucial approach to create healthy workplace environment and maintain professionals’ mental well-being.

Methodology

Study Design and Sampling Plan:

Comparative study design using a convenience sample of 300 healthcare professionals for both groups (150 in the study group and 150 in the comparative groups). Health care providers who participated in this research study were selected regardless of their gender group, specialty, or work time. This strategy was used to clarify the general characteristics of healthcare providers of either group. About 400 healthcare providers received the questionnaire and agreed to be a part of the study; however, only 310 subjects responded to the questionnaire (150) subjects from the first group and (160) subjects from the comparative group. To make the sample size equal in the both groups, 10 subjects were randomly excluded from the comparative group.

Study instrument:

The General Health Questionnaire (GHQ28) is used to assess the mental health of healthcare providers working in the infectious diseases units and the mental health of the comparative group. This scale was developed as a screening tool to detect those likely to have or to be at risk of developing psychiatric disorders. This screening tool was developed by Goldberg in 1978. It is a 28-items that is measure a four levels scale ranged from (0 to 4 ) respectively; 0 indicates no, 1 indicates less than usual, 2 indicates more than usual, 3 indicates more often than usual. The levels of psychological distress among healthcare providers are determined based on the sum of items scores (minimum score 0 and maximum score 84). The cut score is 23/24 and individuals with higher scores are considered having higher level of psychological distress. This scale is includes four subscales in which that each scale consist 7 items, divided as follow: 1) Items (1-7) measure somatic symptoms; 2) Items (8-14) measure anxiety/insomnia; 3)Items (15-21) measure social dysfunction; 4) Items (22-28) measure severe depression. The Arabic version of the scales had good reliability level, Cronbach’s alpha level (0.89).

Ethical Considerations

Healthcare professionals were asked for voluntary participation. Research objectives, risks, and benefits were explained to participants to help them decide whether to participate or not. After they agreed to be a part of the study, participants were given anonymous questionnaire to maintain participants’ confidentiality.

Results and Discussion

The recent study indicates that there is a statistically significant difference between study group (healthcare providers working in infectious diseases units) and comparative group (healthcare providers working indifferent units) in respect to their mental health. More than half of the healthcare professionals working in the infectious diseases units were symptomatic for psychological distress; whereas, 30% of the comparative group (working in non-infectious units) were symptomatic for psychological distress. It indicates that nurses working in high demand or high risk environment anticipated a greater likelihood of developing depression and stress symptoms as a consequence of the continuous concern about their safety. Mentioned that healthcare professional who work in hospitals settings are more likely to be vulnerable to depression, and the risk is greater than the risk compared to the general population. However, highlighted that healthcare workers who are in contact with communicable diseases are at higher risk for stress, anxiety, and depression than other groups of
healthcare providers. Moreover, the various types of infectious diseases add to the work stress of healthcare professionals and increase their vulnerability to the risk of infection (2,20). A literature review study was conducted on the workplace factors that influence the psychological health of healthcare professionals involved in caring for clients with various infectious diseases. This research discovered that those employees had higher baseline depression and anxiety than those who care for clients with non-communicable diseases. These psychological effects related to the nature of environment as a high risk, loss of social support (isolation), stress attached to their role, and risk perception 1. Health care professionals who had direct contact with the affected patients were at great risk for physical and psychological risks. Even though, providers uses personal protection equipment, they still experience barriers of communication and difficulties in diagnosis and caring process, which extent the time needed for each patients and increase the risk for stress. Working in such conditions indicated considerably higher social isolation and felt considerably more vulnerable to somatic symptom, sever anxiety, depression, and fatigue 7. Analysis of variance (ANOVA) was used to find the differences among healthcare providers in response to psychological disturbances as a result of working in high risk environment. The findings indicate that technicians had the highest mean score than other groups of professionals, which represents their vulnerability to psychological disturbance, followed by physicians and nurses. The study of 17 compared the phenomenon of burnout among healthcare professionals working in the same situation. Researchers indicate a significant difference across professional groups in terms of being subjected to burnout and emotional disturbance. The difference was explained by several factors, including the responsibilities and roles assigned for each group, as well as the type of patients they care for. Being at the frontline in contact with patient can also increase the vulnerability of work stress. In addition, training programs can have positive impacts on reducing work-related stress. Significant relationships were found between psychological health and professionals’ working conditions, including training about infection control, availability of the personal protective equipment, and availability of hand hygiene. Several studies have highlighted the importance of providing training for infection prevention and control, as well as the importance of using personal protection equipment for all healthcare professionals working in the infectious diseases units to prevent the potential risk and reduce work stress 10. The study that carried out by 3 showed that healthcare professionals who had not received infection prevention training were less likely to have knowledge of infection prevention than those who had received infection prevention training. Therefore, lack of knowledge increases their vulnerability to stress. Moreover, training has positive impact on the attitude of the respondents in the study group compared to the control group (16, 3). Health practitioners need to safeguard themselves with barriers such as gloves, face masks, gowns, protective eyewear and face shields to reduce work-related microorganism transmission. Despite that, the threat cannot be secured completely, such as the threat results from needlestick injury, using personal protective equipment (PPE) regularly can protect both the professional and the patient from potentially infectious body fluids 5. The research of 21 linked the increased risk for professionals’ infection to their noncompliance in using hand hygiene before and after caring process. Most providers of healthcare underestimate the significance of hand hygiene as it is not correctly stressed. As a result, many nurses lack the expertise and resources necessary to promote their compliance. Re-education; therefore, enables them by emphasizing the importance of compliance with hand hygiene, which increases the quality and safety of provided healthcare services. In addition, studies have also shown that personal protection equipment are not all available for healthcare professionals; however, even with the availability of required equipment, some professionals are careless to use the protective materials. Therefore, hand hygiene is critical to reduce the risk of infection transmission among patients and prevent the risk for healthcare providers (0,5).

**Conclusion**

This study aims to assess the mental well-being of healthcare professionals working in infectious diseases units and compare these results to the score of other healthcare professionals working in other units. Workplace stress has been found in all healthcare providers as a result of various types of stressor factors, which impact their mental health. However, healthcare providers who are in contact with infectious diseases are at higher risk for depression, stress, anxiety, and sleep disturbance than others who work in different healthcare settings. Consequently, feeling stressed and concerned about personal safety impose a negative effect on professionals’ mental health. More training about infection prevention help raising professionals’
knowledge and practice of preventing infection from being transmitted to other patients or to the healthcare providers. Likewise, using personal protective equipment and hand hygiene play an important role in reducing the risk of infection among healthcare professionals. Therefore, it is important for healthcare professionals to be aware of the effective strategies that reduce their vulnerability to the risk of infection. More training about infection prevention and provide personal protection materials help reducing the risk of disease transmission among patients and to health care providers, which indeed helps reducing the psychological stress among healthcare professionals.

Table 1: psychological disturbance among healthcare professionals: The study group

<table>
<thead>
<tr>
<th>Psychological Status</th>
<th>Overall Psychological disturbance</th>
<th>Somatic symptoms</th>
<th>Anxiety/insomnia</th>
<th>Social dysfunction</th>
<th>Severe depression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F.</td>
<td>%</td>
<td>F.</td>
<td>%</td>
<td>F.</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>73</td>
<td>48.7</td>
<td>66</td>
<td>44.0</td>
<td>66</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>77</td>
<td>51.3</td>
<td>84</td>
<td>56.0</td>
<td>84</td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td>100.0</td>
<td>150</td>
<td>100.0</td>
<td>150</td>
</tr>
</tbody>
</table>

Table 2: Psychological disturbance among healthcare providers: The comparative group

<table>
<thead>
<tr>
<th>Overall Psychological Disturbance</th>
<th>F.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic</td>
<td>106</td>
<td>70.7</td>
</tr>
<tr>
<td>symptomatic</td>
<td>44</td>
<td>29.3</td>
</tr>
<tr>
<td>total</td>
<td>150</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 3: Difference between study group and comparative group in response to psychological disturbance.

<table>
<thead>
<tr>
<th>Independent Samples Test</th>
<th>Levene’s Test for Equality of Variances</th>
<th>'t-test for Equality of Means</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>Sig.</td>
</tr>
<tr>
<td>Equal variances assumed</td>
<td>31.977</td>
<td>.000</td>
</tr>
<tr>
<td>Equal variances not assumed</td>
<td>4.833</td>
<td>252.522</td>
</tr>
</tbody>
</table>
Table 4: Mean scores of healthcare providers in response to psychological distress:

<table>
<thead>
<tr>
<th>Healthcare professionals</th>
<th>Mean</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nurse</td>
<td>.8473</td>
<td>84</td>
</tr>
<tr>
<td>Physician</td>
<td>.8979</td>
<td>21</td>
</tr>
<tr>
<td>Pharmacist and Pharmacist Assistant</td>
<td>.4404</td>
<td>6</td>
</tr>
<tr>
<td>Technicians</td>
<td>1.130</td>
<td>39</td>
</tr>
</tbody>
</table>

*The higher the mean score, the higher the risk for mental disturbance

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of Nursing, University of Babylon, Iraq and all experiments were carried out in accordance with approved guidelines.

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Emotional Intelligence and Work-related Stress among Nurses Working in Psychiatric Hospitals

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¹ MScN, ² PhD, College Nursing/University of Babylon

Abstract

Background: Emotional Intelligence is a broad concept that connecting various personality and cognitive traits of human beings. The concept of emotional intelligence is described as the individual’s capability of recognizing their own emotions and the emotions of others, and practice this understanding to navigate important interactions successfully.

Aims: The purpose of this study is to assess emotional intelligence among nurses working in psychiatric hospitals, as well as the relationship between emotional intelligence and workplace stress.

Methodology: A descriptive correlational study design using a convenience sample of (N=109) nurses working in two main psychiatric hospitals. Two separated scales (The Emotional Intelligence Scale-34 items and The Work-related Stress scale-24 items) was used to meet the study objectives.

Results: The study findings indicate that emotional intelligence levels were ranged between moderate to high among study participants. However, the majority had moderate work-related stress about (61%). Nurses’ work stress was found to have significant relationship with their emotional intelligent level.

Conclusion and Recommendations: Despite the positive levels of emotional intelligence nurse have, working in psychiatric hospitals is still cause of stress for nurses. The statistical relationship found in this study highlights the importance of enhancing emotional intelligence among nurses working in psychiatric hospitals settings by engaging them in effective training programs to maximize their abilities of adjustment and reduce their levels of work-stress.

Keywords: Emotional Intelligence, Work-related Stress, Psychiatric Nurses

Introduction

Emotional Intelligence (EI) is a broad concept that connects different personality and cognitive traits of humans, including emotional processing, emotional understanding, integrating affects and management of feeling states. The concept first introduced by Salovey and Mayer in 1990, and it was defined as cognitive skills that introspectively can detect and regulate affective states and assess the emotional state of others. In general, emotional intelligence is described as the ability of an individual to recognize own emotions and others emotions, and to practice this understanding to navigate important interactions successfully. A clear understanding of EI and its difficulties is helpful in the practice of nursing; due to the understanding of how emotions merge with cognition. Generally, EI helps nurses to improve relationships, manage their patients more effectively, make better decisions, and positively affects the quality of care provided to patients and families. The study aims to assess emotional intelligence among nurses working in psychiatric hospitals, as well as the relationship between emotional intelligence and workplace stress. Emotional intelligence is mainly focus on people with the ability to express, evaluate, and adjust enthusiasms and emotions of themselves and others, as well as those who can properly guide and control the thought and performance. Therefore, emotional intelligence is considered as an effective power to deal with job stressors. Also, nurses with high emotional...
intelligence and emotional managements are able to direct positive feelings exchanged by healthcare individuals to decrease the negative effects of job stress. Nurses experience and suffer from stress and health problems have less traits related of their work characteristics and less contact with patients. Emotions can have an impact on job outcomes and emotional intelligence, which could explain the differences among individuals in dealing with work stress. Emotional intelligence can have a direct influence on the quality of the provided healthcare services or indirect influence, through other mediator, variables such as individual’s job satisfaction and professional conflict. Studies have shown that nurses communication skills and job satisfaction play significant role in the emotional intelligence of nurses and the quality of the provided services. Moreover, work-related outcomes are more likely to be impacted when nurses feel stressed and insecure in their work environment.

**Methodology**

**Study Design and Sampling Plan:**

A descriptive correlational study design using a convenience sample of (N=109) psychiatric nurses working in the main psychiatric hospitals in Baghdad Province. Both male and female nurses were included regardless of their working shift. Their age ranged between 20 – 60 years old, with at least one years of experience in the psychiatric hospital.

**Study instrument:**

Two separated scales were used to meet the study objectives. The first scale is the Revised Emotional Intelligence Scale-(34) items. The original emotional intelligence scale was developed by, which contains (65 items); then, it was reduced to (34 items). The revised scale is widely used to measure the emotional intelligence cross-cultures. This scale is a self-reported instrument that is measured and rated on three levels rating scale; 1= not applicable to me, 2= applicable to some extent, 3= highly applicable. The levels of emotional intelligence are determined based on mean scores of the total items as follow: 1) nurses with means scores of (1 - 1.66) are considered having low emotional intelligence; 2) nurses with means scores of (1.67 – 2.33) are considered having moderate emotional intelligence; 3) nurses with means scores of (2.34 - 3) are considered having high emotional intelligence. The second scale is the Karasek Professional-Related Stress Scale was adopted and modified to be used in this study to assess workplace stress among psychiatric nurses. The original scale was developed by Karasek in 1980. This scale is also a self-reported instrument; however, it is measured and rated on four levels rating scale; “1= strongly disagree, 2= disagree, 3= agree, 4= strongly agree”. Coding for negative statements was reversed for statistical purpose. The levels of work stress are determined based on total scores of the scale items as follow: 1) nurses with total scores of (58 – 69) are considered having high work related stress. 2) nurses with total scores of (70 – 81) are considered having moderate work-related stress; 3) nurses with total scores of (82 - higher) are considered having low level of work-related stress.

**Scale Validity and Reliability**

The Revised Emotional Intelligence Scale and The Karasek Professional-Related Stress Scale were translated to Arabic by two bilingual content experts. The Arabic versions of both scale were presented to panel of content experts related to the fields of mental health, psychology, and community health to maintain the validity of the Arabic versions. Both scales had good reliability levels with a Cronbach’s alpha level (0.89) for the emotional intelligence scale and a Cronbach’s alpha level (0.78) for the work stress scale.

**Ethical Considerations**

Nurses were asked for voluntary participation. Research objectives, risks, and benefits were explained to participants to help them decide whether to participate or not. After they agreed to be a part of the study, participants were given anonymous questionnaire to maintain participants’ confidentiality.

**Results and Discussion**

This study indicates that majority of the study sample (60.6%) had high level of emotional intelligence, and (39.4%) had moderate level. The results of this study show that female nurses have higher level of emotional intelligence, than male nurses. Recently, there are some studies have been conducted to study nurses’ emotional intelligence in several fields. Empathy, emotional awareness, and problem solving were the most common attributes of emotional intelligence that have been reported by nurses. Researchers discovered that the nurses’ emotional intelligence was higher than other professionals. This difference is related to the fact that nurses get more training about caring process and
Another research was conducted in the Netherlands showed higher emotional intelligence scores among mental health nurses compared to the general population. Engaging in more ethical behavior have been indicated by nurses who reported high levels of emotional intelligence. Moreover, it has been found that the high level of emotional intelligence among clinical nurses play a role in organizational justice, specifically regarding interpersonal and informational aspects of relationships. The outcomes of this study show moderate level of work-related stress was identified in (60.6%) of psychiatric nurses; while (14.7%) had high level of stress related to their workplace. Nursing personnel are considered as professionals with increased level of stress, anxiety, and depression due to their daily routine, which is particularly demanding since they are forced to cope with pain, sadness, and death. Stress also comes from the fatigue resulted from an increased workload, as well as the inability of nurses to cope with the emotional needs of patients and their families. Nurses who work in psychiatric settings are more likely to experience work violence than other nurses who work in different healthcare settings. Violence is negatively impact the provided quality psychiatric care and increase the level of stress among nurses. Shu-Fen Niu et al. (2019) have highlighted alarming percentages about the experienced violence by nurses that cause higher level of work-related stress among nurses population. Researchers indicate that more than 50% of the psychiatric nurses involved in their study experienced physical violence and more than 80% experienced psychological violence by psychiatric patients. Resilience of nurses is another concept could explain the work-related stress among psychiatric nurses participated in this study. Most recent systematic review study was conducted on 38 articles that targeted nurses resilience and work-related factors. Researched pointed out that “Job demands (stress, burnout, posttraumatic stress disorder, and workplace bullying) were negatively associated with resilience, while job resources (coping skills, self-efficacy, social support, job satisfaction, job retention, and general wellbeing) were positively related to resilience.” A significant inverse relationship between emotional intelligence and work-related stress was identified among psychiatric nurses, in which that stress decreased when emotional intelligent increased. Several studies have highlighted the link between emotional intelligence and work-related stress among nurses. Nurses who have a high level of emotional Intelligence build relations within an organization and with the people they serve; whereas, those with low level of EI may tend to create problems for the organization through their individual behaviors. Therefore, developing emotional intelligence should be emphasized in order to overcome stress at workplace and accomplish goals and cope with stress. The study of represent that emotions play an important role in a profession that requires not only technical expertise, but mainly relies on the psychological and physical care of human beings. The positive effects of EI with respect to the quality of nursing work and the ability to mitigate the impact of a difficult working environment via the contribution of emotional self-management and the personal stress management capacity in environments that is full of stress. EI enables nurses to effectively manage and deal with stress at work. Furthermore, the ability of individuals to regulate emotion can have positive effect on group cohesiveness and that the emotional intelligence level of team leaders can improve team empowerment and minimize stress among workers. It has been found that longer careers, job retention, and participation in clinical ladder programs are related to higher emotional intelligence scores of clinical staff nurses. Moreover, there is relationship between emotional intelligence and nurse reports of self-compassion that affect positively on their performance, higher nurse emotional intelligence is associated with lower levels stress and less burnout. In addition, less confrontational anxiety was associated with the ability to regulate emotion.  

**Conclusion**

The recent study aims to measure the phenomenon of emotional intelligence among nurses working in psychiatric hospitals and the relationship with their stress at work. The findings show that more than half of nurses working in psychiatric hospitals had high emotional intelligence level. However, despite the positive results, working in psychiatric hospitals is still a source of stress for nurses. The statistical relationship appears in this study between emotional intelligence and workplace stress highlights the importance of enhancing EI among nurses, as it improves the capacity to exercise skills in a manner that benefits patients, families, peers, and themselves as practitioners. Furthermore, increased emotional intelligence can lead to more successful management, job satisfaction, organizational commitment, better patient health outcomes, and less workplace stress.
Table 1: Emotional intelligence levels among psychiatric nurses

<table>
<thead>
<tr>
<th>Emotional Intelligence Levels</th>
<th>F</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate emotional intelligence</td>
<td>43</td>
<td>39.4</td>
</tr>
<tr>
<td>High emotional intelligence</td>
<td>66</td>
<td>60.6</td>
</tr>
<tr>
<td>Total</td>
<td>109</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2: Work-related stress among psychiatric nurses

<table>
<thead>
<tr>
<th>Work-related Stress levels</th>
<th>F</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild stress</td>
<td>27</td>
<td>24.8</td>
</tr>
<tr>
<td>Moderate stress</td>
<td>66</td>
<td>60.6</td>
</tr>
<tr>
<td>High stress</td>
<td>16</td>
<td>14.7</td>
</tr>
<tr>
<td>Total</td>
<td>109</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 3: Relationship between emotional intelligence and work-related stress

<table>
<thead>
<tr>
<th>Dependent and independent variables</th>
<th>Emotional Intelligence</th>
<th>Work-related Stress</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pearson Correlation</td>
<td>.345**</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>109</td>
</tr>
<tr>
<td>Emotional Intelligence</td>
<td>Pearson Correlation</td>
<td>.345**</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>109</td>
</tr>
<tr>
<td>Work-related Stress</td>
<td>Pearson Correlation</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>109</td>
</tr>
</tbody>
</table>

Table 4: Regression analysis to predict work-related stress from nurse’s emotional intelligence.

<table>
<thead>
<tr>
<th>Dependent &amp; Independent Variables</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>t</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Constant)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emotional intelligence</td>
<td>.354</td>
<td>.345</td>
<td>3.807</td>
<td>.000</td>
</tr>
</tbody>
</table>

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Nursing/University of Babylon, Iraq and all experiments were carried out in accordance with approved guidelines.

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The Relationship Between Smoking and Urokinase gene 3’-UTR T/C Expression on Occurrence of Bladder Cancer

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1Department of Biology, College of Education for Pure Sciences, University of Kerbala, Kerbala, Iraq,
2College of Medicine, University of Kerbala, Kerbala, Iraq

Abstract

Bladder cancer (CA Bladder) is the malignancy that affects the lining of the bladder (which is the most common types of urinary epithelial cancers that associated with the most invasive types of cancers and has the highest incidence of recurrence and infections). This study was designed to investigate the role of cigarette smoking on expression of urokinase gene that has a prominent role in the incidence of CA bladder. The samples were collected from 90 patients after being clinically diagnosed by the specialist surgeon of Imam Hussein Center for Cancer Diseases, Holy Kerbala, Iraq who were heavily smokers (more than one pocket/day). These patients were compared to 90 non-smoker patients with CA bladder and 90 persons who apparently healthy individuals as a control groups, DNA was extracted from all blood samples. The level of gene expression was correlated with cycle threshold value calculated by using real time PCR.

The results showed significant association between smoking and occurrence of CA bladder (by increased expression of urokinase 3’-UTR T/C gene) in comparison to both control and non-smoker patients groups.

Keywords: CA bladder DNA, cigarette smoking, Urokinase gene 3’-UTR T/C, real time PCR.

Introduction

The genetic investigations for polymorphisms help to distinguish between the different inherited forms of the gene and their impact on the occurrence of many human diseases include cancer 6. The extent of the risk factors like smoking and genetic predisposition of many affect the occurrence of many diseases such as cancer, heart disease and diabetes 1. The urokinase-type plasminogen activator uPA system is a responsible for transforming plasminogen into plasmin, which has different physiological functions 10. It additionally assumes a key signaling protein in disease intrusion and metastasis both locally and spread away for far destinations 4.

The urokinase gene is located at chromosome 10q24 9. Polymorphisms of a C/T transversion at the 3’ UTR (+4065 nucleotide) were demonstrated by C/T substitution in exon 6 and a T/C substitution in intron 7 8. Among the risk factors that participate in the occurrence of CA bladder, smoking was detected as one of major one 3. High levels of urokinase have been depicted in bladder tumors especially in those who were heavily smokers 5.

Methodology

Blood samples were collected from males of age group ranging from 50-60 years old by the Imam Hussein Center for Cancer and Hematology at the Imam Hussain Teaching Hospital in Holy Karbala province, Iraq. The samples were include a 90 heavily smoker patients after the clinically determination of bladder malignancy (transitional cell carcinoma) by authorized specialist, 90 patients with CA bladder with no history of smoking for at least the past 25 years, and 90 apparently healthy non-smoker individuals as a control group. Both patients and control groups were matched regarding age, body weight, past medical history and any associated confounding factors. This study was extended for period from January to August 2018. DNA was extracted from all blood samples by DNA extraction
kit (Bioneer,Korea). Polymerase chain reaction (PCR) was adopted for amplification of gene, The premiers were Designed by STS Accession No. G27040: forward primer 5'-CCGCAGTCA- CCAAGGAAGAG-3'and backward primer 5'GCCTGAGGGGTAAGCT-ATTGTCGTGCAC-3'.

The RT-PCR protocol involve the following steps: (a) initial one cycle of denaturation for 5 minutes at 95°C, (b) followed by annealing at 58°C for 40 seconds, (c) then extension at 72°C for 40 seconds, (d) with final step of holding the specimen at 8°C. Real time PCR was used for quantitative assessment of urokinase gene 3'-UTR T/C expression by measuring cycle threshold (cT) value (which is inversely proportional to the level of gene expression).

Statistical Analysis

Data were statistically represented as mean±SD and analyzed by detection of variance of significance using ANOVA test by sigma plot software version 12.5.

Results and Discussion

Smoking was thoroughly studied as a predisposing factor for many human disease include cardiovascular disorders, respiratory diseases malignancies, neurological deficits and many other organic dysfunction. In current study, the aim was to demonstrate the

Table (1): the cT value of urokinase gene 3'-UTR T/C (expressed as Mean±SD) estimated by real time PCR.

<table>
<thead>
<tr>
<th>Group Name</th>
<th>No.</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA smokers</td>
<td>90</td>
<td>12.58±2.692</td>
</tr>
<tr>
<td>CA non smokers</td>
<td>90</td>
<td>23.21±3.569</td>
</tr>
<tr>
<td>healthy non smokers</td>
<td>90</td>
<td>24.86±2.861</td>
</tr>
</tbody>
</table>

The difference in cT values were quite significant between smoker patients as compared to both non-smoker patients and non-smoker healthy persons (12.58±2.692 versus 23.21±3.569 at P<0.001 and 12.58±2.692 versus 24.86±2.861 at P<0.001 respectively). On the other hand, no significant difference was detected between non-smoker patients in comparison to non-smoker healthy individuals (23.21±3.569 versus 24.86±2.861). These findings suggested that smoking has potential inducing effect for expression of urokinase gene 3'-UTR T/C which has crucial role in occurrence of CA bladder in heavily smokers. Other finding was noticed that the occurrence of CA bladder in non-smoker patients may be attributed to other sort of predisposing factor or gene.

This association was mentioned in certain study, which suggested that chronic exposure to cigarette smoking had strong association with development of urothelial carcinoma.

In another study done to evaluate the effect of smoking on soluble 3'-UTR isoforms(regulatory pathway of bronchial epithelial cell function), similar results were founded supporting the potential role of smoking as predisposing factor for CA bladder in our study.

This effect of smoking was explained in certain study as a result of the effect of smoking on p53 pathway that mediate apoptotic signaling that responsible for eradication of cancer cells.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Education for Pure Sciences, University of Kerbala, Kerbala, Iraq and all experiments were carried out in accordance with approved guidelines.

References


Effect of Carcinogenic Substance (7,12 Dimethylbenz [a] anthracene (DMBA)) on Tissue, Hematology Character and Enzyme Activity in Rat

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Abstract
This study aimed to investigate the effects of 7,12 dimethylbenz [a] anthracene (DMBA) on some blood parameters and hepatic histopathology in rats and antioxidant enzyme. Twenty female Wistar albino rats, weighing 180–200 g, were randomly divided into two group. DMBA group (positive group) who received 20 mg DMBA/kg body weight/ (single Douse). Control group (negative group) don’t received any thing. The animals in the groups were sacrificed at the end of the 90 days: The histological structure of the liver tissues in the control group was normal. the liver exhibited hydropic degeneration and coagulation necrosis in hepatocytes, severe dilation in the sinusoids, congestion in the central and portal regions, DMBA groups, was degenerative and necrotic changes were detected. MDA levels increased in the DMBA group, compared to the control group, Antioxidant activity CAT, SOD and GPX, the results showed increased enzyme activity in positive control compared with negative groups, the hematological parameter was increase in WBC, PLT, GRA, and LYM and decrease in RBC value. Keywords: 7,12-dimethylbenz[a]anthracene (DMBA); Blood parameters; Antioxidant enzyme ; MDA ; Rat.

Introduction
Cancer, also termed as malignant neoplasm, is a type of diseases where in a group of cells show abnormal proliferation, invasion and sometimes metastasis. Cancer begins when cells in a part of the body start to grow out of control. There are more than 100 types of cancer have been identified. The substances that promote cancers are called carcinogens, and agents that have the ability to change DNA in ways that are inherited by daughter cells are called mutagens. Most cancers are related to environmental, lifestyle, or behavioral exposures. The term “environmental”, as used by cancer researchers, refers to everything outside the body that interacts with humans. Other chemicals, like benzene, ketones, vinyl chloride, ethylene bromide, and dichloro-diphenyl-trichloroethane (DDT), are known carcinogens. The accumulation of multiple factors, carcinogens and altered genes, transform a normal cell into a cancerous one. Carcinogens that have the ability to promote cancer have various sources. The factors responsible for cancer development are classified as exogenous and endogenous. The first group includes nutritional habits (food preservation and preparation), socio-economic status, lifestyle, physical agents (ionising and non-ionising radiation), chemical compounds (natural and synthetic) and biological agents (Helicobacter pylori, Epstein Barr virus, human T lymphotropic viruses I and II, human papilloma virus and the hepatitis B virus).

Material and Method
Experiential animals and Tumor induction in rats:
Twenty female of Wistar albino rats and aged between (7-8) weeks were weighing between 150 – 250 g used in this study. All they were kept in ventilated cages, with temperature of 25±2C. A 12:12 h light:dark cycle is also regulated for these animals. Balanced rodent food pellet and water is provided. The rats were randomly assigned to 2 groups of 10 rats each.

The groups were as follow:
Group 1: Received a single dose of DMBA (positive control) 20 mg DMBA/kg body weight/ (single dose)

Group 2: Did not received any treatment (negative control).

DMBA administration: Mammary tumors were induced by 7, 12-

Dimethylbenz (a) anthracene (DMBA) 10 . A single dose of DMBA dissolved in corn oil was given by oral gavage to two groups using the syringe and needles. DMBA was purchased from Sigma Aldrich and dissolved in corn oil. The concentration of the solution was 20 mg DMBA per 1 ml corn oil for each rat 10 .

Preparation of tissue sample

The liver was exteriorized and excised. All specimens were immediately fixed in 10% formaldehyde solution. After fixation they were processed in usual manner, and embedded in paraffin for subsequent histopathological examination for liver. A scoring system (of no abnormalities, mild, moderate, severe) was used to classify the liver changes according to the severity of the damage and extent of histological changes. The histological sections were evaluated by a pathologist without prior knowledge of the treatment given to the animals 11 .

Blood parameters were determined in whole blood by the using rat mode of veterinary practice with a blood cell counter (Abocus Junior Vet-5, Austria). Measurements of biochemical parameters were made with a Modular PP autoanalyzer (Mindray BS800, China).

Results

Histopathological findings: The control group showed normal histological structure in the liver tissue (Figure 1A). In the DMBA group, the livers exhibited dilatation of the sinusoids, cholangiohepatitis in the portal region, and congestion in the sinusoidal and portal regions (Figure 1B). Hematological parameters also change in negative group compared of positive group Table 1 , in positive group : white blood cell (WBC) , LYM, MON,GRA and PLT while other parameter such as RBC was decrease. The state of free radicals and antioxidants is given in Table 2. As seen in Table 2, the levels of MDA increased in liver groups compared to the control group, also SOD, GSH-Px, CAT, and GSH values decreased.

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameter</th>
<th>Negative control</th>
<th>Positive control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>WBC (103 /mm3 )</td>
<td>8.2</td>
<td>15.1</td>
</tr>
<tr>
<td>2</td>
<td>RBC(103 /mm3 )</td>
<td>8.8</td>
<td>1.4</td>
</tr>
<tr>
<td>3</td>
<td>LYM %</td>
<td>67%</td>
<td>78.5 %</td>
</tr>
<tr>
<td>4</td>
<td>MON%</td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>5</td>
<td>GRA %</td>
<td>18.8 %</td>
<td>94 %</td>
</tr>
<tr>
<td>6</td>
<td>MCV(fl)</td>
<td>56.6</td>
<td>58.7</td>
</tr>
<tr>
<td>7</td>
<td>MCH(Pg)</td>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td>8</td>
<td>MCHC(g/dl)</td>
<td>32.3</td>
<td>32.8</td>
</tr>
<tr>
<td>9</td>
<td>PLT(105 /mm3)</td>
<td>338</td>
<td>737</td>
</tr>
</tbody>
</table>
Table 2. Some biochemical parameters in the groups (values are mean n=4)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Conc. of GPX (IU/mg)</th>
<th>Conc. Of SOD (IU/mg)</th>
<th>Conc. of CAT (IU/mg)</th>
<th>Conc. of MDA (nmol/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative group</td>
<td>127</td>
<td>458</td>
<td>122</td>
<td>134</td>
</tr>
<tr>
<td>Positive group</td>
<td>312.3</td>
<td>567</td>
<td>187</td>
<td>267</td>
</tr>
</tbody>
</table>

Discussion

7,12-dimethylbenz(α)anthracene (DMBA) is a well known carcinogen and immunosuppressor used in rodent models to study cancer. DMBA is reported to induce mutations by making DNA adducts. Although, it is a well known skin carcinogen, yet many researchers have reported the deleterious effect of DMBA in liver. Liver is the primary site of metabolism and is often prone to damage by xenobiotics. Evidently, liver cancer is the second most common cause of cancer deaths worldwide. Several haematological and haematochemical parameters were changed when treated with DMBA and found that DMBA induced hepatocellular carcinoma. Experimental studies showed that DMBA-induced skin, oral, mammary and ovarian tumors. The carcinogenic and mutagenic effect of DMBA requires its metabolic activation by mixed function oxidases. The hydroxylation of DMBA at 7-methyl group is a crucial step towards its carcinogenesis. Further metabolism of DMBA leads to formation of a wide range of metabolites with varying toxicity. Among these, trans-3,4-dihydrodiol-1,2-epoxide is the carcinogenic product of DMBA. The metabolic products of DMBA, when present inside body, hampers ROS-antioxidant balance by overproduction of free radicals and the body in turn reacts by modulating activities of antioxidant enzymes to curb the damaging effects of an increased ROS. Hematological and biochemical parameters may be affected by a variety of factors such as race, age, gender, pregnancy, lactation, muscular activity, region, season, environmental heat, maintenance, and nutrition. In the present study, the effects on blood parameters and hepatic, histopathology of fluoride. Oxidative products derived from mutagen metabolism, such as DMBA, might impair vital cellular function by damaging proteins and lipid membranes. Consequently, these changes induced by the chemical carcinogen 7,12-dimethylbenz[a]anthracene, have been reported to be leukemia, and the development of anemia. Reactive oxygen species (ROS) are important as pathological agents for many diseases. Increased oxygen radical production and lipid peroxidation are associated with the pathogenesis of many diseases and the toxic effects of a wide range of compounds increase in total leukocyte count (WBC), eosinophil, neutrophil and monocyte values for rats exposed to DMBA was also reported. Hematological and biochemical parameters may be affected by a variety of factors such as race, age, gender, pregnancy, lactation, muscular activity, region, season, environmental heat, maintenance, and nutrition. A significant increase in the GPX, CAT, SOD level in rat treated with DMBA only was reported. On other hand find MDA content increase significantly with rat treated with DMBA only.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Nursing, University of Warith Al-aniyia’a, Iraq and all experiments were carried out in accordance with approved guidelines.

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Psychological Factors Affecting on Developing Reading Skills and Evaluation of Reading Strategy Knowledge

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Abstract

The questions are one of the most common and used methods in the educational process and in schools all over the world and are the most important educational strategies. Because questions are a great place in teaching, which is the mainstay of the dialogue method and is like the driving force in the lesson. The educators say (If you want to become a highly competent educator, it is important that you begin to apply and develop educational questions). These questions also occupy great importance in the educational process after which the standard means of knowing the readiness of the students and the level of information they received, in a good question, we achieve the desired goal and achieve a desirable educational product. Besides, the exams are a means to help educators to make different educational decisions, so that, there have been wide concerns calling for the reform of the test questions and the evidence of that conferences that discussed this problem, studies and research aimed at diagnosing them in order to address, improve and develop their defects to achieve best results in education.

Key words: evaluation, Arabic Language Questions, six Grade Students, Standard Specification Table.

Introduction

Education is the basis for the reform of humanity and its success. It is a force that can develop individuals, sharpen their talents, sharpen their minds and ideas. The education of the individual means comprehensive education that is integrated in all aspects, spiritual, mental, physical, psychological, social and aesthetic. 1

The method of education is the language that benefits the individual in understanding cultural aspects is the means of understanding and the means of education and the collection of cultures, a tool for the transfer of ideas, but is a tool of thinking and sense and feeling. 2 In summary: Language is one of the tools of public life, and it does not perform its duty unless fulfills its purpose for, and does not reach its real status with its people unless they are concerned about what they are and about. 2 Through this way, the Arabic language is one of the most important elements of our Arab nation. It is the language of the Holy Quran, which says: “We have sent down Arab villages.” (2) As the Quran absorbed the characteristics of the language and it’s all stylistic features and its characteristic expression until tomorrow, the basis for which they control the validity of the language and the reverberation in the knowledge of its secrets and facts. 3

Thus, Arabic is one of the most important elements of our Arab nation and by which the nation held fast and boycotted the sermons. As well as it is the language addressed by God Almighty Prophet Muhammad (peace be upon him and peace) as it is the language that came down the Koran by saying: (We sent down Arab villages that you may know) ( Yousif / 2). The Holy Quran absorbed the characteristics of the language and it’s all stylistic features and its characteristic expression Until become the basis for the validity of the language and the state return to know the secrets and facts. 6

The Arabic language is a branch of a large group called the Semitic languages, and the first to be known as the German orientalist Schlutzer in 1781. 3 The evaluation of exam questions is a component of the curriculum, because it is the process of ensuring the achievement of goals, the relationship between the evaluation process and the educational goals, the evaluation process aims at knowing the extent to which these goals are achieved, or the extent of their progress towards the educational goals to be achieved. The importance of the evaluation is clear because of its great impact in the process of change and development in the educational process, because on the basis of a sound objective evaluation can be achieved a lot of educational principles, in addition to showing and treating weaknesses and showing positive aspects and
1. Evaluation: “It is the last stage that depends on measurement and decision-making, and then the feedback that makes teaching and teaching circular rather than linear”.

2. Questions: The field in which the student acquires knowledge and mental skills and intellectual capacities and works on their development and development.

3. Exam: A systematic objective method for measuring a sample of a person’s skills or abilities or a group of individuals in a particular time and place.

4. Bloom classification: A classification that contains a range of public areas, which include the possible educational data, and the expected educational process.

The theories and models of evaluation are very numerous and difficult to define each model that has many positives that make it suitable under conditions. And certain educational programs, and no matter how many calendar models, for the good qualities that should be characterized by any model of the calendar and any measure or any test are:

1. The Objectivity: - Objectively means that the procedures of evaluation and its components or measurements are accurate without falsehood away from self-impact and personal impressions of the transient to be true and consistent.

2. It is appropriate that the evaluation be appropriate in its tools, procedures and results for the subject of evaluation.

3. The Inclusion: - that the evaluation process is comprehensive so that the study programs or the subject to be evaluated in all its aspects and not by a certain aspect of it.

4. The Stability: that the evaluation process results in the same results if the results are re-analyzed or the application of measurement and evaluation tools is repeated.

5. The Honesty, ie, the evaluation means what is intended to evaluate it cannot study something and measure something else.

6. The Easiness: - That the evaluation and its tools process can be easily applied within the available material and human resources available and under the local conditions designed for them. The researcher will discuss the classification of Bloom and the criteria adopted in the analysis of the questions of Arabic grammar for sixth grade students. The scientific and literary branches in detail in the third chapter.

**The Results of the study are as follows:**

1. The questions of reading subjects in the primary stage are markedly concerned with the level of understanding and remembering. The percentage of questions of comprehension (47.3%) and the level of remembering (40.8%). The levels of application, analysis, installation and evaluation were very small.

2. - The questions of reading topics are concerned with the skills of direct understanding of the text, and the skills of understanding the conclusion of a greater interest in higher thinking skills such as the skill of criticism and taste, the proportions were respectively: (57.6%), the deductive understanding (34.5%), the critical comprehension (6.9%), and the final taste (1.7%). The test questions focused on the questions of understanding the direct meaning by 68% and the deductive questions by 32%.

3. Reading comprehension questions (50.3%), comprehension level (31.1%) and application (9.1%), followed by the remaining levels at low rates.

4. The predominant type of questions in subjects of reading is the most important questions (34.8%), while the balance of questions is (51.4%) compared with (48.6%) for the objective questions.

**The research methodology and procedures:**

It includes a presentation of the procedures that have been carried out to achieve the research objectives, starting with the research methodology and descriptive design, defining the research community and its design, preparing the research materials and tools, and presenting the statistical means used.

The Descriptive Design: The researcher followed descriptive research methodology to analyze the content of test questions because it is the appropriate method for analyzing questions, which is one of the research methods used in education and psychology. The aim of this approach is to provide data and facts about the problem of the subject to be interpreted and to identify...
its implications.

**The research community and design:**

The research society includes the general (ministerial) examination questions for the Arabic grammar for the third grade in the Republic of Iraq for the academic years of the academic year (2014/2015) to the academic year (2018/2019), and the first two and the third and the number of (11) exam paper, (5) papers for the first round and (5) exam papers for the second round an examination paper for the third round and as Annex 1, as the research community represents the questions of the ministerial examinations of the sixth scientific grade therefore, the whole research community will be a basic sample that will be adopted by the researcher in his procedures.

**Table (1) shows the research community and its sample:**

<table>
<thead>
<tr>
<th>No.</th>
<th>Academic year</th>
<th>No. of main questions</th>
<th>No. of sub-questions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2014-2015 attempt /1</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>2014-2015 attempt /2</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>2014/2015 2014-2015 attempt 3</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>2015/2016 2014-2015 attempt 1</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>2015/20162014-2015 attempt 2</td>
<td>7</td>
<td>18</td>
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<td>6</td>
<td>2016/2017 2014-2015 attempt 2</td>
<td>7</td>
<td>19</td>
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<tr>
<td>7</td>
<td>2016/2017 attempt2</td>
<td>7</td>
<td>20</td>
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<tr>
<td>8</td>
<td>2017/2018 attempt 1</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>9</td>
<td>2017/2018 attempt 2</td>
<td>7</td>
<td>22</td>
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<td>10</td>
<td>2018/2019 attempts 1</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>11</td>
<td>2018/2019 attempts 2</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td>77</td>
<td>223</td>
</tr>
</tbody>
</table>

The research Tool:

**A - Sources of building the tool:**

The structure of the criteria for the evaluation of the ministerial examinations of Arabic grammar through:

- Building an open survey questionnaire addressed to teachers and specialists in the methods of teaching Arabic language and measurement and evaluation.

- See a collection of previous studies and literature relevant to the current research topic that examines the standards of good questions. By means of exploratory resolution and the sources of tool construction, the orthodontic criteria that analyzed the test were built. The study consisted of six criteria and these criteria were analyzed by the questions of the ministerial examinations of the grammar of the Arabic language for the sixth grade in the scientific branches on the grounds that these criteria are the most important criteria that should be available in the examination questions are:

  - Independence.
  - Direct and indirect questions.
  - Objectivity.
  - Inclusion.
  - Bloom’s levels of knowledge.
  - Technical aspects.
B - Tool credibility:

These criteria were presented to a sample of experts and arbitrators with expertise and experience for the purpose of identifying the validity and importance of each paragraph of the questionnaire, in order to achieve the apparent honesty. A standard was established to measure each paragraph (valid, not valid, observations). In this respect, Ebel points out that the test is true if its verbs measure what has been measured.

The researcher relied on the arbitrators to indicate the validity of the paragraphs of the questionnaire in their precise reference to the paragraphs, which is an indicator of the apparent authenticity of the questionnaire, and after collecting the questionnaires from the arbitrators, the researcher determined the proportion of agreement (90%) and more than the opinions of experts on the validity of the paragraph, because it represents the view of the majority, Agreement as follows:

- Independence 100%
- Objectivity 100%
- Bloom levels of the field of knowledge 100%
- Inclusive 100%
- Direct and indirect questions 95%
- Technical aspects 97%

In addition, some experts and arbitrators suggested that another criterion be increased, namely the proportion of correspondence to the textbook questions. The criteria were as follows:

- Independence
- Objectivity
- Bloom’s levels of knowledge
- Inclusion
- Correspondence to textbook questions
- Direct and indirect questions
- Technical aspects.

Steps to analyze the test questions:
A - Reading the contents of my book Arabic grammar rules for the sixth grade scientific reading good to know all content and topics.

B - Reading the questions a good reading and answer them as appropriate.

C) The question which contains several branches, each of which is treated as an independent question.

D - matching the questions to the criteria adopted by the researcher.

E) Unloading the results of the analysis into the criteria for analyzing the questions.

Evaluation criteria for ministerial examination questions:

There are scientific educational foundations that must be available in any exam to trust its results and benefit from it. The good exam is the one that is fit to perform the purpose for which it was set up in the fullest manner. Such a test will not be complete unless there is information on its validity for measurement.

Bloom’s Levels of Knowledge:

Many of the studies in Bloom’s classification (1956) indicate that this classification is one of the most important works that can help to analyze questions in general and in a procedural manner to contain all levels that reflect the desired objectives of the education process.

The Bloom classification is one of the most important attempts to address behavioral goals and the most widely used. It has an advantage in the identification and identification of educational goals.

Bloom’s classification helps teachers gain a clear idea of the patterns of behavior emphasized by a particular set of educational schemes. As well as it represents an educational system and benefit for all teachers, administrators and those interested in education and researchers who are in the Department of specialization in the issues of curriculum and evaluation.

Therefore, the researcher chose this classification because it is one of the most common classifications because it contains a very wide range of behavioral patterns of goals that most educational programs and systems are expected to achieve. It also benefits educators in general, and teachers who seek to define their educational goals in special behavioral terms.

Those who are interested in measurement and evaluation, led by Bloom, set behavioral goals in three areas. A meeting of the University of Chicago in 1956 resulted in an agreement to classify goals into three areas:

(Cognitive / mental, emotional / emotional, and motor).
Statistical means:

The researcher used the following statistical means:
- percentage
- Equation (scott) to find the stability of analysis.

Conclusion

The ministerial examinations are of great importance, because it represents the boundary between the stage and the other, especially since each stage characteristics and advantages differ from the stage that precedes it, especially the transition from secondary level to university; The current research problem revolves around the validity of ministerial examination questions for the Arabic language for the sixth scientific grade and the extent to which the characteristics of good questions. In the light of the criteria standard and the analysis of questions according to this standard. The importance of the current research stems from the importance of the Arabic language and its importance to all the subjects, the importance of the ministerial examinations also stems from the fact that they are the most important evaluation tools currently used in our schools.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Babylon / college of Basic Education, Iraq and all experiments were carried out in accordance with approved guidelines.

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Comparison between Photoselective Vaporization of Prostate by Diode Laser and Monopolar Transurethral Resection of Prostate in Single Center Experience

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Abstract

Background: Transurethral resection of prostate has been regarded as the gold standard surgical management of benign prostatic hyperplasia (BPH). With the evolution of new technologies, laser prostatectomy emerged as an alternative.

Aim of the study: To compare the rate of outcome of diode laser vaporization of prostate and Transurethral Resection of Prostate in Al Diwaniya governorate.

Patients and methods: From October 2017 to October 2018, forty patients with surgical indications of prostatectomy were enrolled in this study. Their ages ranged from 60-75 years with a mean age 68.6 years and prostate size ranged from 40-80 ml with a mean size 62.175 ml. Twenty patients underwent diode laser vaporization of prostate and another 20 patients underwent Transurethral Resection of Prostate. The choice based on surgeon preference and patient ability. Preoperative evaluation was done for all patients by history, physical examination and investigations. International Prostate Symptoms Score, maximum flow rate, digital rectal examination, prostate specific antigen, abdominal ultrasound, urinalysis, blood urea, s.creatinine, complete blood count were all done preoperatively. One pint of compatible fresh blood prepared.

Results: The rate of significant bleeding and transfusion in diode PVP and TURP was (zero vs. 35% respectively), in PVP and TURP the mean irrigation amount perioperatively was (10.10 vs. 33.15 pints respectively), mean operative time was (70.80 vs. 50.00 min respectively), mean hospital stay was (16.03 vs. 30.09 hours respectively), mean time to urethral catheter removal was (24 vs. 81.71 hours respectively), postoperative complication rate (mainly dysuria) was (75% vs. 65% respectively), mean postoperative IPSS was (14.40 vs. 14.20 respectively), mean postoperative Qmax was (15.45 vs. 16.30 ml/sec respectively). There was no statistical difference between both groups in regard to lowering IPSS or increment in Qmax. The mean cost was (1500 vs. 500.42 dollars) in diode PVP and TURP respectively.

Conclusion: Diode laser vaporization of prostate is a good alternative to TURP with comparable results in treatment of BPH especially in patients taking anticoagulant drugs who cannot stop their medication. However, the high cost of this procedure and dysuria as a complication may prevent the common use of this technique.

Key word: Photoselective vaporization of prostate, Transurethral resection of prostate, Benign prostatic hyperplasia.

Introduction

Benign Prostatic Hyperplasia (BPH) is a histologic diagnosis that refers to the proliferation of smooth muscle and epithelial cells within the prostatic transition zone.
BPH is commonly characterized by lower urinary tract symptoms (LUTS). The prevalence of histologically diagnosed BPH increases from 8 percent in men aged 31 to 40, to 40 to 50 percent in men aged 51 to 60, to over 80 percent in men older than age 80. The prevalence of moderate or severe LUTS for men in the fifth, sixth, seventh, and eighth decades of life to be 26, 33, 41, and 46 percent, respectively. Patients often present with LUTS as storage irritative symptoms (frequency, urgency and nocturia) due to bladder response to obstruction (increased pressure) and voiding obstructive symptoms (straining, weak stream, intermittency and feeling of incomplete bladder emptying). BPH is a progressive condition that can lead to serious long-term complications such as acute urinary retention (AUR), renal insufficiency, development of gross hematuria, bladder calculi, urinary incontinence, and recurrent urinary tract infections (UTIs). Medical history can establish the severity of LUTS by IPSS. PSA should only be performed if life expectancy is greater than 10 years and if a diagnosis of prostate cancer would modify the management approach. Medical treatment includes Alpha 1 antagonist, 5-alpha reductase inhibitors, Anti-muscarinic agents and Phosphodiesterase-5 inhibitors, when medical treatment fails to control patients' complaints, surgical intervention is mandatory; however, surgery is associated with a number of complications. Surgical approach includes TURP. Laser prostatectomy is either coagulation or vaporization (or both), resection or enucleation depending on the wavelength applied. The usage of TURP is gradually decreasing (nearly 5%/year). Types of laser procedures includes HoLEP which can be used in large glands (100-150) and has comparable efficacy and good outcome compared to open prostatectomy and TURP but longer operating time. In this study, we want to compare the rate of intraoperative bleeding and the need for blood transfusion, the amount of irrigant, operative time, hospital stay, time of urethral catheterization, postoperative complications and the cost of diode laser vaporization of prostate and TURP in Al Diwaniya governorate.

Patients and Methods

From October 2017 to October 2018, forty patients with surgical indications of prostatectomy were enrolled in this study. Their ages ranged from 60-75 years with a mean age 68.6 years and prostate size ranged from 40-80 ml with a mean size 62.175 ml. Twenty patients underwent diode laser vaporization of prostate and another 20 patients underwent Transurethral Resection of Prostate. The choice based on surgeon preference and patient ability. Preoperative evaluation was done for all patients by history, physical examination and investigations. International Prostate Symptoms Score, maximum flow rate, digital rectal examination, prostate specific antigen, abdominal ultrasound, urinalysis, blood urea, s.creatinine, complete blood count were all done preoperatively. One pint of compatible fresh blood prepared.

Results

Our patients data were comparable between the two groups with a mean age of 68.6±4.14 years (range 60-75 years) and mean prostate size of 62.175 ± 10.885 ml (range 40-80 ml). Their mean PSA was 3.615 ng/ml (range 1.5-8 ng/ml). Regarding vesical stones, one patient (5%) of laser group was with 18 mm stone and 3 patients (15%) of TURP group with 18, 13 and 15 mm vesical stones which were removed before beginning of the procedure. Four patients (20%) were on anticoagulants who could not stop their medications (all of them on warfarin 5 mg, 2 patients with cardiac stents, 1 patient with prosthetic mitral valve and 1 patient with deep lower limb venous thrombosis). All of them were enrolled in laser group as shown in table 1.

Table 1: General characteristics of patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Laser n = 20</th>
<th>TURP n = 20</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year) mean ±SD</td>
<td>68.50 ±4.14</td>
<td>68.70 ±4.03</td>
<td>0.878* NS</td>
</tr>
<tr>
<td>Size of prostate (ml) mean ±SD</td>
<td>59.35 ±10.885</td>
<td>65.00 ±9.03</td>
<td>0.082* NS</td>
</tr>
<tr>
<td>PSA (ng/ml) mean ±SD</td>
<td>3.62 ±2.07</td>
<td>3.62 ±1.72</td>
<td>0.993* NS</td>
</tr>
<tr>
<td>Presence of stones &lt; 20 mm, n (%)</td>
<td>1 (5%)</td>
<td>3 (15%)</td>
<td>0.598 † NS</td>
</tr>
<tr>
<td>Patients on anticoagulant, n (%)</td>
<td>4 (20%)</td>
<td>0 (0%)</td>
<td>0.106 ¥ NS</td>
</tr>
</tbody>
</table>

n: number of cases; SD: standard deviation; *: independent samples t-test; †: Yates correction for continuity; ¥: Fischer exact test; NS: not significant.
The indications for intervention in both groups were the same and included thirteen patients (32.5%) with moderate to severe IPSS (who were already on medical treatment), recurrent urinary retention in 10 patients (25%), gross hematuria in 4 patients (10%), recurrent urinary tract infection in 10 patients (25%) and deterioration in renal function in 3 patients (7.5%).

**Table 2: indications of surgery**

<table>
<thead>
<tr>
<th>Indication</th>
<th>Laser group</th>
<th>TURP group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate – sever IPSS</td>
<td>7 (35%)</td>
<td>6 (30%)</td>
</tr>
<tr>
<td>Recurrent urinary retention</td>
<td>5 (25%)</td>
<td>5 (25%)</td>
</tr>
<tr>
<td>Gross hematuria</td>
<td>2 (10%)</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>Recurrent UTI</td>
<td>4 (20%)</td>
<td>6 (30%)</td>
</tr>
<tr>
<td>Deterioration in renal function</td>
<td>2 (10%)</td>
<td>1 (5%)</td>
</tr>
</tbody>
</table>

The mean operative time in laser group was 70.8 min. (range 60-80 min.) , while in TURP group was 50 min. (range 40 – 60 min.) (figure 11). Regarding intraoperative complications, no patient developed significant intraoperative bleeding in laser group. In our study, we determined significant intraoperative bleeding as reduction in blood pressure that required transfusion, while seven patients (35 %) in TURP group developed significant intraoperative bleeding and required intraoperative blood transfusion. No patient in laser group developed obturator reflex in comparison with TURP group in which 3 patients (15 %) developed obturator reflex, as shown in table 3.

**Table 3: operative time and intraoperative complications**

<table>
<thead>
<tr>
<th></th>
<th>Laser group</th>
<th>TURP group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean operative time(min.)</td>
<td>70.80 ± 5.07</td>
<td>50.00 ± 6.19</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Intraoperative bleeding</td>
<td>0 (0 %)</td>
<td>7 (35%)</td>
<td>0.008 *</td>
</tr>
<tr>
<td>Obturator reflex</td>
<td>0 (0%)</td>
<td>3 (15%)</td>
<td>0.598*</td>
</tr>
</tbody>
</table>

**: independent samples t-test. *: Fischer exact test . SD :standard deviation . HS :highly significant . NS :not significant
Table 4: postoperative care

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Laser group</th>
<th>TURP group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean amount of irrigation (pints) ±SD</td>
<td>10.10±2.59</td>
<td>33.15±5.24</td>
<td>&lt; 0.001 *</td>
</tr>
<tr>
<td>Mean hospital stay (hours) ±SD</td>
<td>16.03±3.89</td>
<td>30.09±10.32</td>
<td>&lt; 0.001 *</td>
</tr>
<tr>
<td>Mean time of urethral catheterization (hours) ±SD</td>
<td>24</td>
<td>81.71±10.34</td>
<td>&lt; 0.001 **</td>
</tr>
<tr>
<td>Mean cost (dollars) ±SD</td>
<td>1500</td>
<td>500.42±195.32</td>
<td>&lt; 0.001 **</td>
</tr>
</tbody>
</table>

*Independent samples t-test. **One sample t-test; SD: standard deviation; HS: highly significant at P ≤ 0.01.

The mean follow up period was two months. All of the patients were assessed clinically by calculating IPSS and Q max. The mean pre-operative IPSS was 17.65 (range 13 – 27) and significantly decreased post operatively in both groups (mean IPSS 14.3 with range of 9-18), with the mean IPSS of laser group decreased from 20.1 to 14.4 and that of TURP decreased from 18.75 to 14.2.

Table 5: IPSS and Q-max before and after operation

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Laser n = 20</th>
<th>TURP n = 20</th>
<th>P *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>IPSS (pre-operative)</td>
<td>20.10</td>
<td>4.30</td>
<td>18.75</td>
</tr>
<tr>
<td>IPSS (post-operative)</td>
<td>14.40</td>
<td>3.05</td>
<td>14.20</td>
</tr>
<tr>
<td>P †</td>
<td>&lt; 0.001 HS</td>
<td>&lt; 0.001 HS</td>
<td>-----</td>
</tr>
<tr>
<td>Q-max(ml/s) (pre-operative)</td>
<td>10.30</td>
<td>1.66</td>
<td>10.20</td>
</tr>
<tr>
<td>Q-max (ml/s) (post-operative)</td>
<td>15.45</td>
<td>1.15</td>
<td>16.30</td>
</tr>
<tr>
<td>P †</td>
<td>&lt; 0.001 HS</td>
<td>&lt; 0.001 HS</td>
<td>-----</td>
</tr>
</tbody>
</table>

n: number of cases; SD: standard deviation; S: significant *: independent samples t-test; †: Paired samples t-test; NS: not significant at P ≤ 0.05; HS: highly significant at P ≤ 0.01.

Regarding post-operative complications: no patient in laser group had frank hematuria in the immediate post-operative period compared to 2 patients (10 %) in TURP group. TUR syndrome did not occur in neither group. Four patients (20 %) in laser group had UTI in comparison to 5 patients (25 %) in TURP group. Acute retention occurred in 3 patients (15 %) in each group. Eight patients (40%) in laser groups complained from dysuria compared to 3 patients (15%) in TURP group. The overall complications rate was 75 % vs. 65 % for laser and TURP groups respectively. As shown in table 6.
Table 6: Post-operative complications

<table>
<thead>
<tr>
<th>Complication</th>
<th>Laser n= 20</th>
<th>TURP n =20</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-operative bleeding</td>
<td>0 (0%)</td>
<td>2 (10 %)</td>
<td>0.487 *</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>TUR syndrome</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>---</td>
</tr>
<tr>
<td>UTI</td>
<td>4 (20 %)</td>
<td>5 (25 %)</td>
<td>1.000 †</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Acute retention</td>
<td>3 (15 %)</td>
<td>3(15 %)</td>
<td>1.000 †</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Dysuria</td>
<td>8 (40 %)</td>
<td>3 (15 %)</td>
<td>0.077**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NS</td>
</tr>
</tbody>
</table>

n: number of cases; *: Fischer exact test; †; ¥: Yates correction for continuity; **: Chi-square test; NS: not significant at P ≤ 0.05

Discussion

In this study, we compared the rate of intraoperative bleeding, the amount of irrigation fluid needed, operative time, hospital stay, time to urethral catheter removal, postoperative complications, follow up results and the cost. In our study, no patient in laser group developed significant bleeding, while in TURP 35% of patients had significant bleeding and needed transfusion. Kuntz RM reported that bleeding and need for transfusion is reduced significantly in diode laser PVP compared to TURP. 19 Erol A. et al reported 1 patient with diode PVP developed bleeding that needed catheterization and irrigation. 20 Seitz M. et al stated no patient had significant bleeding in diode PVP. 21 Our study is comparable to these results. Reich O. et al reported rate of 7.1% of blood transfusion in TURP in 10,654 patients, 22 while in our study it is 35 %. This high rate may be due to small sample study. The amount of irrigation needed for laser group is much less (mean 10.10 pints) than TURP group (33.15 pints) and this is because less encountered bleeding in laser group. Mithani MH. et al had mean of 16.44 pints for laser group. 23 In our study, the operative time of laser group is longer than that of TURP by about 20 minutes. Razzaghi MR also reported higher operative time of laser because the time it takes for the light energy absorption by water and Hb. 24 In our study, the mean hospital stay for laser and TURP groups was 16.03 and 30.09 hours respectively. This less stay for laser can be attributed to less bleeding and uncomplicated procedure.

Conclusion

Diode laser vaporization of prostate is a good alternative to TURP with comparable results in treatment of BPH especially in patients taking anticoagulants who cannot stop their medication and this advantage make it better than TURP. However, the high cost of this procedure and dysuria as a complication may reduce the common use of this technique.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Al-Diwaniyah teaching hospital/ department of urology/ Al-Diwaniyah province, Iraq and all experiments were carried out in accordance with approved guidelines.
References


Tympanometric Findings among Adults with Chronic Nasal Obstruction Due to Sinonasal Pathology

Salim Hussain Ibrahim
Department of surgery-Otorhinolaryngology Head and neck surgery; Tikrit Medical College –Iraq

Abstract

**Background:** The nasal obstruction is one of the causes that affect the middle ear function, but the physiological mechanism of this effect is not well understood. This study aims at 1st; evaluation of the middle ear function among adults with chronic nasal obstruction due to sinonasal pathology using tympanometry, and 2nd, at comparison between the anatomical and mucosal inflammatory causes of nasal obstruction and its impact on the middle ear function.

**Patients and Method:** A prospective case study of (310) adult patients with chronic nasal obstruction were subjected to tympanometry for study of middle ear pressure in the private ENT clinic for a period of two years in the Salah Al-Deen governorate.

**Results:** this study found that (13%) of adults with chronic nasal obstruction had abnormal tympanogram (type B or C), (17.2%) was due to mucosal inflammation and (7%) due to anatomical and structural cause. Commonly one ear affected (unilateral) was (67.5%). Myringotomy and aspiration of middle ear found that (64%) had OME and VT was inserted.

**Conclusion:** The study statistically proved that adults with chronic nasal obstruction due to mucosal inflammation differs from others due to anatomical and structural causes in the followings; 1. The incidence of abnormal tympanogram (type B and C) is more. 2. The type B (OME) was more than type C (Eustachian tube dysfunction). 3. In the treatment, it was found that the need to VT insertion was more.

**Keywords:** Tympanometry, Chronic nasal obstruction, Otitis media with effusion, Nasal polyposis, Chronic rhinosinusitis.

Introduction

One of the major ENT complaints is chronic nasal obstruction. A definite relation between nasal obstruction and middle ear diseases, where Eustachian tube dysfunction (ETD) caused by nasal obstruction, that may cause middle ear hypoventilation resulting in otitis media with effusion (OME), that may be definitely diagnosed through use of otoscopic findings associated with a B tympanogram (1,2,3). Some previous researches have been carried out on how the nasal obstruction that influence middle ear function, but the physiologic impact of this effect is not well defined (4,5,6). The evaluation of the middle ear function with a high rate of success in the management and follow up of middle ear diseases can be carried out by Tympanometry, because of its compliance, flexibility of the ear drum to changing air pressure 7. This study aims at 1st; evaluation of the middle ear function among adults with chronic nasal obstruction due to sinonasal pathology using tympanometry, and 2nd, at comparison between the anatomical and mucosal inflammatory causes of nasal obstruction and its impact on the middle ear function.

Patients and Method

A prospective case study of 310 adult patients with chronic nasal obstruction (>3 months) were subjected to tympanometry (620 ears) for study of middle ear pressure in a private ENT clinic during a period from 1st January 2017– 1st January 2019, in the salah El-den governorate. Iraq. Male were 184 (59%) and female were 126 (41 %), age ranging from 18–63 years (mean age...
The diagnosis of nasal pathology was based on detailed history taken, proper ENT examination includes nasal endoscopy and investigation includes computed tomography of the nose and paranasal sinuses. Ear examination includes otoscopic examination, Tuning fork tests. Tympanometry, PTA when abnormal tympanometry. Exclusion criteria includes nasoapharyngeal mass or tumor, recent history of acute rhinitis or middle ear infection, chronic suppurative otitis media, previous nasal or ear surgery, tympanosclerosis, no case with nasal malignancy. Patients were classified according to the causes of nasal obstruction into:

1. Patients with nasal obstruction due to anatomical and structural causes includes Septal deviation, hypertrophied turbinates, concha bullosa, nasal valve collapse, synchia, choanal atresia.

2. Patients with nasal obstruction due to Mucosal inflammation, such as allergic rhinitis with or without nasal polyposis (NP), non-allergic rhinitis, and chronic rhinosinusitis (CRS).

Tympanometry was carried out using viola middle ear analyzers from inventis audiology equipment / Italy to conduct automatic tympanometry tests at low frequency (226 Hz).

The tympanometer probe was inserted into the clean ear canal. The types of tympanogram are classified according to Jerger/Fiellau-Nikolajsen in to the following:

1. Type A is a normal peaked curve with pressure between +50 and -99 daPa, Compliance from 0.3-1.5 ml. Suggests normal middle ear functioning

2. Type B is a flat curve without peak, Compliance less than 0.3 ml suggests middle ear effusion

3. Type C is a peak curve with negative pressure (-100 or more negative), compliance from (0.3-1.5 ml). Suggest Eustachian tube dysfunction.

Results

From total 310 patients with chronic nasal obstruction 40 patients (13%) had abnormal tympanogram (type B or C). (Table 1). An abnormal tympanogram was found in patients with chronic nasal obstruction due to the mucosal inflammation was 31/180 patients (17.2%), and in patients due to anatomical causes was 9/130 patients (7%), Which is statistically significant association p-Value 0.018. (Table 1). Patients complaining of nasal obstruction due to mucosal inflammation the type B/C tympanogram were as follows, in allergic and non-allergic rhinitis was 7/57 patients (10.5%), nasal polyposis (NP) was 17/94 patients (18%), and in chronic rhinosinusitis (CRS) was 7/29 patients (24%), anatomical and structural causes 9/130 (7%) as shown in (Table 2). Patients who had an abnormal tympanogram in one ear (unilateral) were 27/40 patients (67.5%) and in both ears (Bilateral) were 13/40 patients (32.5%) (Table 2). Regarding the type of tympanogram, the normal type A was in 567/620 ear (91.5%), type B was 25/620 ear (4%), and type C was 28/620 (4.5%), this statistically very strong association p-value =0.005 (Table 3).

Patients who underwent surgical treatment were 31/40 patients (77.5%) (9 patients refused to carry out the operation or were missing). Total number of ears who have abnormal tympanogram was 39 ear (8 patients were in both ears and 23 patients in one ear). Those patients have undergone myringotomy and aspiration of middle ear and found that 25/39 ear (64%) fluid was aspirated (OME) so that a VT was inserted. Shah VT was inserted in 23/39 patients (59%) and Good T-tube in 2/39 patients (5%) who had adhesive otitis media, this results was statistically significant association P-value =0.05 (Table 5). The study found that the Shah VT was inserted in 23/33 ear (70%) of patients with chronic nasal obstruction due to mucosal inflammation, and in 2/6 ear (33%) of patients with anatomical causes. (Table 4) The research found that 24/40 (60%) of Patients who had abnormal tympanogram were complaining from hearing loss or fullness of ears and tinnitus and (40%) of patients deny the presence of such ear symptoms.
Table 1: Relation between causes of nasal obstruction and abnormal tympanogram.

<table>
<thead>
<tr>
<th>Pathological causes of nasal obstruction</th>
<th>Patients No</th>
<th>Abnormal tympanogram</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anatomical and structural causes</td>
<td>130 (42%)</td>
<td>9 (7%)</td>
</tr>
<tr>
<td>Mucosal inflammation</td>
<td>180 (58%)</td>
<td>31 (17.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>310 (100%)</td>
<td>40 (13%)</td>
</tr>
</tbody>
</table>

$X^2=5.58$, P.value=0.018, d.f.=1

Table 2: The relation of sinonasal pathology and abnormal tympanogram.

<table>
<thead>
<tr>
<th>Sino-nasal pathology</th>
<th>Patients No (%)</th>
<th>Abnormal tympanogram</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Unilateral</td>
</tr>
<tr>
<td></td>
<td>310(100%)</td>
<td>40 (13%)</td>
</tr>
</tbody>
</table>

* Includes ;Septal deviation ,hypertrophied turbinates, concha bullosa, nasal valve collapse. Congenital choanal atresia.

** Includes ;Antrochoanal polyp.

Table 3: The relation between causes of nasal obstruction and type of tympanogram.

<table>
<thead>
<tr>
<th>Pathological causes of nasal obstruction</th>
<th>Ear No</th>
<th>Type of tympanogram</th>
<th>Type A</th>
<th>Type B</th>
<th>Type C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>260</td>
<td>249 (95.8%)</td>
<td>4 (1.5%)</td>
<td>7 (2.7%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>360</td>
<td>318 (88%)</td>
<td>21 (6 %)</td>
<td>21 (6%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>620</td>
<td>567 (91.5%)</td>
<td>25 (4%)</td>
<td>28 (4.5%)</td>
<td></td>
</tr>
</tbody>
</table>

$X^2=10.77$, P.value=0.005, d.f.=2

Table 4: Relation between the causes of nasal obstruction and surgical procedure on the ear.

<table>
<thead>
<tr>
<th>Causes of nasal obstruction</th>
<th>patients</th>
<th>Ear No</th>
<th>Surgical procedure</th>
<th>Grommet insertion</th>
<th>Grommet insertion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Myringotomy only</td>
<td>Shah VT</td>
<td>T-tube</td>
</tr>
<tr>
<td>Anatomical &amp; structural causes</td>
<td>5</td>
<td>6</td>
<td>4 (67%)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Mucosal inflammation</td>
<td>26</td>
<td>33</td>
<td>10 (30%)</td>
<td>21</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>39</td>
<td>14 (36%)</td>
<td>23 (59%)</td>
<td>2 (5%)</td>
</tr>
</tbody>
</table>

$X^2=2.9$, P.value=0.05, d.f.=1
The incidence and severity of middle ear diseases has been found to be related to the cause of nasal obstruction, whether anatomical and structural causes or mucosal pathologies. There are several suggested theories about the effect of nasal obstruction on the Eustachian tube, one theory suggest that nasal obstruction leads to turbulence of air flow in the nasopharynx. The turbulent air flow may leads to Eustachian tube dysfunction (ETD) by the following supposed mechanisms. 1. Dryness of mucosal film leads to increased viscosity of the mucous and the surface tension at the tubal orifice interfering the opening of the Eustachian tube. 2. The air pollutant and microorganisms deposited in the ET orifice causing tubal or pre tubal inflammation and obstruction. 3. Stimulation of the mechanoreceptors, leading to reflex alteration of (ET) function. Many studies have been conducted to assess the correction of nasal obstruction due anatomical causes and the study of its impact on the middle ear dysfunction. Nanda. et al, & Osama G. et al have found that septoplasty improve hearing and middle ear function by improving ET function, but Şahin, et al found that no effect of septoplasty on middle ear pressure and ET function. The whole mucosa of the upper respiratory tract, including the nasal cavity, sinuses, nasopharynx, eustachian tube, and middle ear, which belong to a system called the rhino-pharyngo-tubal unit or the “unified airspace”. Therefore, the presence of inflammatory disease that affects both the nasal and middle ear mucosa is considered the more likely physiologic mechanism that leads to middle ear diseases.

The mucosal inflammation in Allergic rhinitis, NP and CRS contribute to congestion, including increased venous engorgement, nasal secretions from nasal glands and tissue swelling (edema), the accumulated secretion and edema block the eustachian tube. This can reduced middle ear ventilation leading to negative middle ear pressure. There is a significant association between OME and persistent symptoms of allergic rhinitis, these results suggest a direct involvement of the middle ear mucosa. Parietti-Winkler et al. they reported an association between OME and nasal polyposis. They concluded that the mechanism causes of OME in NP and CRS is more likely the presence of inflammatory disease that affects the nasal and middle ear mucosa, and less likely the obstruction of the ET by polyps or by congestion of the nasopharyngeal mucosa. There is a study concluded that the OME seem to be an inflammatory disease that extends to the middle ear rather than a consequence of the nasal disease itself. Because even when the symptoms of nasal obstruction are well controlled, the OME can develop or persist.

The current study found that patients with CRS and NP are at greater risk of developing abnormal tympanogram (24%) and (18%) respectively (Table 2), as found by other authors Rennie et al., study on Eustachian tube dysfunction found that 69% were due to CRS with NP, 28% CRS without NP, and 14% due to allergic rhinitis. In CRS the muco purulent or purulent Secretions pass over the pharyngeal end of Eustachian tube and it can lead to inflammation of ET and hypertrophy of tubal tonsil results in ET obstruction. Patel et al (2016) found that symptoms of ET dysfunction are frequent in CRS, and respond well to endoscopic sinus surgery. The current study found that the VT was indicated in 70% of patients with sinonasal mucosal inflammation because of glue like aspirate of the middle ear (OME). While in the anatomical causes VT was indicated in (33%). This results goes with Mary, et al who found that by, where they found that patients with severe CRS with NP are at greater risk of developing OME, and often highly viscous middle ear effusion.

Limitation in this study that combined pathology may be found commonly septal deviation and/or hypertrophied turbinates with allergic rhinitis, nasal polyposis or chronic rhinosinusitis. In this study they were classified with mucosal inflammation causes. This study statistically proved that adults with chronic nasal obstruction due to mucosal inflammation differs from patients with nasal obstruction due to anatomical and structural causes in the followings; 1. The incidence of abnormal tympanogram (type B and C) is more. 2. The type B (OME) was more than type C (Eustachian tube dysfunction). In the treatment, it was found that the need to VT insertion was more. This study has the following recommendations recommendation: It is preferable to do tympanometry for patients with chronic nasal obstruction, especially when it is due to NP and CRS, even if they not complain of hearing loss, tinnitus or fullness in the ear.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols
were approved under the Tikrit Medical College, Iraq and all experiments were carried out in accordance with approved guidelines.

References


The Impact of Mindfulness Education on Elementary School Students

Rawaa Saadoun Al-Abedi¹, Ibtisam Al-Zaywaini¹, Rassem Ahmed Al-Jerayi¹
¹University of Babylon / college of Basic Education, Iraq

Abstract

The current research aims at evaluating the reading for the fourth grade of primary school in the light of the habits of mind and followed the researcher descriptive approach to suitability the current research procedures to achieve the research objective, the researcher prepared a list that included the criteria of habits of mind based on this researcher prepared a questionnaire (6) standards and (141) paragraphs after verifying the authenticity of the tool from its presentation to a group of arbitrators the stability of the analysis using Holistic equation (0.83) The tool consists of (128) paragraphs distributed in six areas The researcher has developed three alternatives (valid), (invalid), (need to be modified), and the researcher used the appropriate statistical means for their research. The data obtained from the analysis process are: Holistic equation, centimeter weight, square cay, and weighted mean.

Keywords: Evaluation, Arabic Reading Book, Habits of Mind

Introduction

The students are the real wealth of the nation and society must be prepared for life, including the development of writing and reading skills, the maximum extension of the hand of reform is the book must be a systematic evaluation of the curriculum to learn the extent to which the goals set for them ¹ Reading is very important in the elementary stage as a natural entry point for learning and primary school fails miserably if it fails to teach its students to read because the student’s success and progress in all subjects depend on his literacy ability is not material with content that can be taught separately from other subjects But is a fundamental part of every other material. It has become clear that there is a need for something new if schools are to start from a traditional mindset that is focused on capacity only so that students can have a kind of mind-set to create a productive self-fulfilling life and therefore the need for effective and effective mental habits such as the need to develop educational goals that reflect The belief that ability is a repertoire of skills that the individual stores and continues to be constantly expanding as one can increase the skill of thinking through man’s efforts. Education in its general form is a familiar process for all reflect an activity produced by human societies across many times and places. It is not only a reflection of social, economic and political life, but it is an important tool in the development and formation of this life, including from different social systems the prosperity of nations and the attainment of their goals are linked to their concerns. These nations raise and nurture a strong generation to take responsibility for the leadership of the nations and hand them over from fathers to sons, therefore, attention to education means providing the right direction and congratulating the environment for the emergence of righteous individuals. Language has a great role in the twenty-first century because it is a new world in which information prevails in all spheres of life as the individual is able to get the information he needs as quickly as possible in the least effort and language is the basic goal through which he knows what he wants ¹. One of these languages is the Arabic language that every speaker has the right to be proud of and is magnified by our Arabic language, which is characterized by the beauty of its methods and the strength of its expression and its vast wealth of words and meanings. reading is the means of the numbers of the individual scientifically and morally is a means of personal harmony and social assistance to the individual to autonomy and adaptation with others and reading helps to quickly understand and gain desired attitudes and behaviors. The textbook offers opportunities for the teacher to use many of the teaching methods, especially those that need to have a textbook in the hands of the student when the teacher assigned a student or several students to read a paragraph of
the book to develop information or a comparison with external information is the basic premise in front of students to the world of research, knowledge, thinking and knowledge.

**Methodology**

The researcher followed descriptive research methodology to analyze the book of Arabic reading for the fourth grade of primary school because it means describing the facts to identify the data related to a particular educational phenomenon and follow closely and analyze and interpret and investigate the relationships and connotations and then access to the explanatory and complementary instructions can be used. The researcher relied on the analytical descriptive approach because it is a method that is compatible with the nature of the research and its requirements. The descriptive approach is a method for studying scientific phenomena or problems through scientific description and then reaching logical explanations with signs and bar Yen and that research is descriptive of a wider use of research in educational and psychological field it is usually looking at the present and diagnose existing phenomena and the relationships with the discovery by classifying them and their works on the interpretation of those phenomena.

**Identify the research community and its sample:**

The research community is identified and identified by the researcher to define their research community as well as to define their own identity so that the current research community is determined the fourth grade reading book in the Republic of Iraq, the tenth edition 1437/2018. Karim Obaid Al Waeli and Dr. Abdul Abas Abdul-Jassim and Turki Abdul Ghafoor Al-Rawi ,the book consisted of (147) page excelled by the researcher’s title book title , the exclusion of these parts of the content is familiar to previous studies and does not affect the analysis procedures directed towards the content related to the objectives of the book and its subjects, which is the subject of physical and moral construction of the student and has the ability to achieve educational experiences, information and facts Concepts, values and trends. Here is a description of this book:

**A table showing the content of the reading book, the number of pages, their sequence and percentages**

<table>
<thead>
<tr>
<th>No.</th>
<th>Contents</th>
<th>Pages no.</th>
<th>Its no.</th>
<th>No. of excluded pages</th>
<th>The remain no.</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Title and author page</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.68</td>
</tr>
<tr>
<td>2</td>
<td>Scientific supervisor page</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0.680</td>
</tr>
<tr>
<td>3</td>
<td>Introduction</td>
<td>3-4</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1.360</td>
</tr>
<tr>
<td>4</td>
<td>Books’ subjects</td>
<td>5-145</td>
<td>140</td>
<td>0</td>
<td>140</td>
<td>97.27</td>
</tr>
<tr>
<td>5</td>
<td>Book contents</td>
<td>146-147</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1.136</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1-147</td>
<td>147</td>
<td>7</td>
<td>140</td>
<td>100%</td>
</tr>
</tbody>
</table>

**The Research Tool:**

As regards to lack of a ready and appropriate tool to gather information commensurate with the purposes and objectives of the research, the researcher adopted the questionnaire as a tool for research and to prepare this tool, the researcher followed the following steps:

- **List habits of reason:**

In order to reach the list of habits of mind and use that list in the analysis of the book of reading for the fourth grade of primary The researcher adopted the following steps:

1 - Review previous studies on the habits of reason
2 - Access to literature and books that dealt with the habits of mind
3 - The preparation of the preliminary list of the habits of reason based on the researcher’s knowledge of past efforts
4 - Directing an open survey questionnaire for supervisors and teachers of the Arabic language in
primary schools, including the following questions:

- What habits should be read in the fourth grade reading books? Please specify in terms of relevance to the primary stage

- Do you think that the book of Arabic reading meets the habits of mind appropriate for the primary stage?

5. Distribution of the preliminary list of the habits of the mind to a group of specialists in the Arabic language to analyze them and determine whether the principles and their sub-indicators belong to the domains received and have been compiled and studied and used to reach the final list.

The tool’s credibility:

Honesty is one of the important characteristics of educational tests and standards psychological and different and is one of the old concepts so it is subjected to a lot of modification and change as a result of the spread and the expansion of the movement of tests and standards in the various humanities and the truth of several types of the researcher has used what fits the nature of her research which is the apparent truthfulness of the initial examination of the content of the instrument and is reached by the consensus of the arbitrators’ assessment of the measure of the instrument to which it was prepared and to achieve the credibility of the tool based on the credibility of arbitrators and specialists in Arabic language and literature and curricula and methods of teaching as well as those who teach Arabic reading for the fourth grade of primary school.The researcher presented the tool in its initial form in order to ascertain the apparent honesty. The arbitrators expressed their observations about the tool and took the researcher. The researcher has adopted (80%) of the opinion of the arbitrators on the validity of the paragraphs, this is indicated by Bloom, as 80% and more of the arbitrators are evidence of an investigation virtual honesty.

The Analysis:

A – The analysis unit: The researcher used the unit of thought in its analysis of the book of Arabic reading, if it is one of the most important units of analysis and the largest and may be the unity of the idea or phrase contains the idea that revolves around the subject of analysis.

B- The analysis steps:

1- Reading each topic of the Arabic reading book for the fourth grade of the primary to identify the ideas contained in the subject in general

2 - Second reading of each line in the subject for the purpose of reaching the phrase or sentence that contains a particular idea

3 - The identification of the phrase containing a specific idea added by the researcher to the principles prepared in advance and work to determine the suitability of this idea with each paragraph of this principle in a special analysis form

4 - Give a repeat of each paragraph correspond to the idea extracted

5 - Calculate the frequencies obtained by each paragraph in one principle and then calculate the frequencies of each principle individually.

The stability analysis:

While the method of content analysis is a scientific tool, there is a need to satisfy the conditions of objectivity, and achieving objectivity requires knowledge of the stability of the analysis. In terms of consistency, the results obtained from applying the analysis tool to the same sample are not changed and one of the most prominent methods of verification of the stability of the most common analysis of the presentation of a sample of the analyzed article on a group of experts and specialists and the people of know-how and experience in the analysis of content.

So the researcher used two experts who have knowledge in the analysis of the content given each of them a random sample and 10% of the analyzed material from the topics of the book of reading and the researcher calculated the correlation between them and the two experts was the correlation coefficient between them and the first analyst (92,0) And between them and the other analyst (85,0%)

The statistical means:

The researcher used the program (spss) statistical means to analyze the results as follows:

1 - The weighted average to measure the extent to which each concept of the questionnaire

2-Square Kai to calculate the honesty of building concepts
3 - Holistic coefficient to calculate the stability of concepts

4-Percent weight to be used in interpreting the results.

Results

The researcher reached the following results: Taking into account the book of Arabic reading of some habits of the mind, which means taking into account individual differences between students. The habits of the mind give a wide range in the educational process to help the teacher to understand the minds of students and enhance their role in providing opportunities for students to learn and think in different ways. The planning and preparation of lessons through the habits of the mind left a great impact on the students, which led to an understanding of the students to read and assimilate well.

Conclusion

The researcher has developed three alternatives (valid), (invalid), (need to be modified), and the researcher used the appropriate statistical means for their research. The data obtained from the analysis process are: Holistic equation, centimeter weight, square cay, and weighted mean.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Babylon / college of Basic Education, Iraq and all experiments were carried out in accordance with approved guidelines.

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Prevalence of Bronchiolitis among Hospitalized Children less than Two Years in Babylon Province

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Abstract

Background: Bronchiolitis is one of viral lower respiratory tract infections that constitute a heavy burden to public health in the Pediatric population worldwide, which occurs most frequently before age 2 years in the winter and early spring months and most frequently induced by a viral infection (respiratory syncytial virus).

Settings and Design: A survey descriptive study conducted in Babylon Province through-out the period of “1st January 2019 to 30 March 2019” (three) special hospitals of pediatrics.

Method and Materials: Non-probability “purposive” sample consists of 3374 subjects, which collected through a review of patient records for (2016, 2017, 2018) in the statistical units in Babylon hospitals. The information of data was collected retrospectively through-out the special questionnaire obtained from the patient’s sheets in the statistical unit.

Statistical analysis used: The data are coded and tested by the application (SPSS) through the descriptive and inferential analysis.

Results: The analysis of the study indicated that the majority of the children (62.7%) were aged (1- 6) months old who lived in rural areas, the male percentage was higher than the female. In addition, the study indicated that the prevalence of bronchiolitis progressed during the last three years.

Conclusions: The study concludes that the higher morbidity of bronchiolitis was in 2018, most of them admitted to the hospital in January months. Also, the duration of hospital stay was > 4 days.

Key-words: Epidemiology, Prevalence, Bronchiolitis, children.

Introduction

Respiratory disorder is considered the most frequent reason for hospitalization and illness in children. Bronchiolitis is the most relevant infection of the LRTI during infancy through the 1 year of life. “Respiratory syncytial virus” (RSV) is the main cause of this condition as it is considered a major leading cause of increasing the rate of morbidity and hospitalized patients number and cost. As long as, the epidemiology of RSV in developing countries has identified that the most common viral cause of LRI globally, bronchiolitis constitutes a heavy burden to public health in the Pediatric population worldwide, particularly in the United States, which causes significant morbidity and mortality in infants and young children. Bronchiolitis mortality rate is roughly 2 per 100 000 infants and is higher in developing than in developed countries, and statistical data showed that globally there are 150 million new cases of bronchiolitis annually. Also, it has been reported that hospitalization of children during seasonal Respiratory syncytial virus epidemics about 2-3 % of all infants less than 1 year. In the United States, there is significant season-to-season variation in RSV with the period of the peak prevalence varying by as much as 7 weeks (ranging from early January to late February) between seasons. In Egypt, during the period between “October 2016 and March
2017; found that most of the children 53.3% were diagnosed with bronchiolitis and 31.7% were diagnosed with pneumonia. The median duration of the hospital stay four days. Prophylaxis is considered the most effective and safe method that is used in the prevention of respiratory syncytial virus (RSV) disease in infants.

**Methodology**

The study aims to determining the prevalence of bronchiolitis among hospitalized children less than two years

Study design: A cross-sectional descriptive study that conducted through the period of 1 January 2019 to 30 March 2019.

Setting study: the study conducted in in Babylon province at “Pediatric Teaching hospitals”.

Sample of study: A total of 3374 is selected by a convenience non-probability sample through a review of statistical records which were selected out from the main hospitals in Babylon province.

Study instrument: The information of data was collected retrospectively through-out the special questionnaire obtained through a review of patient records for (2016, 2017, 2018) in the statistical units in Babylon hospitals.

Data Analysis: The data are coded and tested by the application “statistical package of social science (SPSS)(Version 24)”. A descriptive analysis includes frequencies and percentage; inferential data analysis included the correlation coefficient, ANOVA test.

**Results**

![Graph showing prevalence of bronchiolitis disease in Babylon province for the last three years (2016-2017-2018). (Total=3,374)](image)

Figure above clearly indicated that the prevalence of bronchiolitis progressed during the last three years and the peak percent 1,442 (42.7%) of the sample occurred in 2018.
Table (1) Distribution of Bronchiolitis prevalence data from statistical records in the hospital.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-6</td>
<td>2,116</td>
<td>62.7</td>
</tr>
<tr>
<td>7-12</td>
<td>770</td>
<td>22.8</td>
</tr>
<tr>
<td>13-18</td>
<td>313</td>
<td>9.3</td>
</tr>
<tr>
<td>19-24</td>
<td>175</td>
<td>5.2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>3,374</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2,051</td>
<td>60.8</td>
</tr>
<tr>
<td>Female</td>
<td>1,323</td>
<td>39.2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>3,374</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>Residency</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>1,420</td>
<td>42.1</td>
</tr>
<tr>
<td>Rural</td>
<td>1,954</td>
<td>57.9</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>3,374</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>Duration of hospitalization stay</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>less than 4 day</td>
<td>2,581</td>
<td>76.5</td>
</tr>
<tr>
<td>≥ 4 days</td>
<td>793</td>
<td>23.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>3,374</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>Month of admission</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>January</td>
<td>2,170</td>
<td>64.3</td>
</tr>
<tr>
<td>February</td>
<td>783</td>
<td>23.2</td>
</tr>
<tr>
<td>March</td>
<td>421</td>
<td>12.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>3,374</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>Outcome of discharge</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well</td>
<td>2,182</td>
<td>64.7%</td>
</tr>
<tr>
<td>Un recover or chronic</td>
<td>542</td>
<td>13.4%</td>
</tr>
<tr>
<td>Dead, un known</td>
<td>740</td>
<td>21.9</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Years of admission</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2018</td>
<td>1,442</td>
<td>42.7</td>
</tr>
<tr>
<td>2017</td>
<td>1,176</td>
<td>34.9</td>
</tr>
<tr>
<td>2016</td>
<td>756</td>
<td>22.4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>3,374</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table (1) reveals that the majority of children were male in the past three years which signified as (60.8%) with (62.7 %) of babies aged less than 6 months; most of them admitted in January and recovered within a short period of hospitalization stay( less than 4 days). The highest percent 57.9 % were lived in the rural area.

The findings in the table above also reported that the prevalence of bronchiolitis was raised in the last three years, particularly in 2018.

**Discussion**

Prevalence of bronchiolitis disease for the last three years (2016-2017-2018)

The findings in the figure (1) reveals that the higher morbidity of bronchiolitis was in 2018. The researcher indicated in the present study that bronchiolitis was increased gradually in the last three years (2016,2017,2018) consequently. This study go along with a retrospective study carried out in Italian about “Comparing of two different epidemic seasons of
bronchiolitis”, which done to evaluate the differences of bronchiolitis frequency and characteristics of the bronchiolitis-related hospitalization between two seasons; S1(2015-16), S2(2016-17). The author indicated that the bronchiolitis morbidity rising from 17% in 2015-16 to 26% in 2016-17. Also, it showed an increased rate of hospitalization11. Another study conducted at Al-Zarqa Government Hospital, Jordan. It’s goal to analyze the epidemiology of RSV infection on 271 children less than 24 months with bronchiolitis, from January 1997 and May 1999. The results indicated gradual rising with peak incidence were in 1999(12). Rationally speaking, Iraqi children’s displacement and migration, particularly during the winter season, live in a crowded situation with bad earnings and health facilities that increased the incidence of communicable diseases with missing or dropping out in the immunization follow-up timetable.

**Distribution of Bronchiolitis prevalence data from statistical records**

In related to the prevalence of bronchiolitis as shown in the table (4.6), the study underhand proved that the majority sample was males aged less than six months who lived in a rural area, admitted in January, 76.5% stayed less than four days in the hospital which increased within two years progressed. a prospective study at Al-Bashir Hospital, Jordan, on children’s admission < 24 months. The author mentioned that the majority sample 60% were male,(53%)were < 6 months, with the annual peak during January and February. In reason, low immunity and high incidence of RSV in children less than 6 months during the winter season. Furthermore, the research showed that the duration of hospital stay was 4 days, the length of stay as indices for the severity of bronchiolitis and linked with absence of breastfeeding, reduced age, greater viral load and sepsis. Certain study indicated that the length of hospital stay was 3 days. The period varied in this study from 4 days in 2010 to 3 days in 2015, and the mean length of stay similar to that reported in Spanish studies13. In the term of discharge outcome in the present study, more than half 64.7% were well status. The outcome discharge and LOS are dependent on effective management. Evidence study conducted at Meherpur General Hospital, about the RSV outbreak to determine the etiology and explore possible risk factors. Which indicated that no death among analysing cases, (36%) Still sick after 5 days of illness and (39%) recovery within 5 days14.

**Conclusion**

The study concludes that predominant age is (1-6) months with a majority to male than female, the higher prevalence of bronchiolitis was in 2018, LOS was less than four days in majority of children, with improved status.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of Nursing, University of Babylon, Hilla City, Iraq and all experiments were carried out in accordance with approved guidelines.

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Nanotechnology Issues Included in the Subjects of the Department of Biology in the Colleges of Education

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Abstract

This research aimed to: Recognize how much including in content of subjects in the Department of Biology College of Education in accordance with the Nanotechnology Issues. To achieve this aim, the researchers built a standard for the Nanotechnology Issues, were verified validity by presentation to a group of arbitrators specialists in the methods of teaching the Biology, and that standard is finalized be from (8) Nanotechnology Issues and emerged of them (90) sub- Issues, then the researchers analyzed the content of subjects of the Department of Biology in Colleges of Education for the academic year (2018-2019) in the light of this criterion as the number of analyst was (12201) page, and it adopted the explicit idea and implicit idea as units for registration and repetition unit of the census, The results were: weakness of subjects in the Department of Biology in Colleges of Education to contain Nanotechnology Issues compared with the ratio based on the opinions of experts (20%), (16) only of 90 terms were achieved, that was equal of (17.77%)

Keywords: Nanotechnology - Nanotechnology Issues - Understanding Students

Introduction

In the midst of rapid and successive developments in most fields of science and knowledge, university institutions and research centers are undertaking these developments as an important tributary to them and a rich resource for their productions in the invention 1, discovery or development, and therefore university education adopts those developments learning and education at the very least, and perhaps the most important science that split its path to global scientific interest is nanotechnology because of the science it has enormous applied fields in various sciences, including Biology, and the first to be the outcome of this science is abundant and based on university students, including students 2 of Biology departments in the colleges of to be educators in the future and need to develop their understanding and awareness of scientific knowledge achievements and provide the latest science and cognitive transformations 3. Technological Despite its many applications, importance, challenges and gravity, it did not receive the required attention from the thought and educational research in Iraq, as it notes the weakness of educational institutions in keeping with the amazing development in this field through its programs and plans 4. Through the information obtained by the researchers from the exploratory questionnaire distributed to a group of teaching specialists in the departments of Biology in the colleges of education and the number (9) include a question on the importance of nanotechnology and the most important issues related to the content of subjects in the departments Biology in the colleges of education, the researchers found that there is a certain desire and urgency of the teachers to address the topics and issues related to nanotechnology because of its global interest in scientific institutions and research centers, and its impact on the intention of the cognitive process for students and their lives, and the above is determined by the following research problem by asking:

Second: The Importance of Research:

Human societies are striving to pay attention to future science in order to face the challenges and developments of the times. Today, our world is witnessing rapid and successive changes as a result of major technological and scientific developments in all fields of life and at all levels. Humanity, and
education, and educational practices allow the learner to take advantage of technological innovations in the development of achievement and skills. Among the technological innovations are nanotechnology which takes care of the study and characterization of nanotechnology materials and their properties at a size less than (100 nm), and precise control of the interaction of molecules and rearrange the atoms and place them in order to produce new materials unprecedented, so that it is possible to design smaller robots from the head of a pin can enter and movement in the blood vessels and be able to perform accurate operations within them and treat blood clots tumors and incurable diseases. 6

Given the importance of nanotechnology in education, many conferences have been held, most notably one, Nanotechnology organized by the University of Jordan in Amman from 10-13 / 10/2008, in cooperation with the University of Illinois at Urbana-Shampeen, under the name of “developed nanomaterials” (14) In Iraq initiatives have emerged related to nanoscience and technology in light of the national education strategy and education that started work formulated and organized since (2009) and launched in (2013) for (10) years, and in this regard published the University of Technology research in the field of nano, and the University of Baghdad with (120) research {15} and given all the above interest in technology Nano and its issues in all fields have emerged many studies and research, including the study Ahmed and others (2015) that sought to investigate the impact of an educational program - learning according to the concepts of renewable energy and know its impact on technological enlightenment in the Department of Chemistry, and showed results the program assists students in acquiring knowledge and concepts related to nanoscience and technology and in technological development. 1

**Nano materials:**

Hijazi defines nanomaterials as “very small materials that are prepared in the laboratory or already present in nature and whose measurements of lengths or diameters of granules range from 0.1 to 100 nanometers.” 2

Forms of nanomaterials: nanomaterials are prepared in various forms including:

The Fullerene: nanoparticles composed of triple bonded carbon atoms, which give the shape of pellets that have a structure similar to graphite, but instead of containing pure hexagonal form, they contain pentagonal forms and potentially sevenfold carbon atoms. 9

Thin Films: a thin layer of a specific material, less than 100 nanometers thick, and their length and width may be in micrometers. These thin layers are used in the field of semiconductors such as silicon and gold bars.

**Nanofibres**

Perhaps the most famous nanofibers are those made of polymer atoms because the ratio of surface area to volume is large in the case of nanofibers, and this gains those fibers characteristic mechanical properties such as rigidity and tensile strength and others, which can be used in biomedicine, and in organ transplantation such as joints, and transport of medicines.

**Nanoballs:**

One of the most important carbon nanoparticles, which ends in the class of Foloreinat, of the material C60, but they differ slightly from the composition as they are multiple crust. 11

**Second: Field Applications in Pharmacy:**

Nanotechnology is one of the most important scientific fields that scientists rely on in the development of drug mutations that change the concepts of treatment and medication for many diseases, and perhaps the most important applications of nanotechnology in pharmacy:

1- Nano-biotics: compounds that have the ability to identify bacteria and viruses, and then eliminate them without exposure to any other cell

2 - delivery of the drug to tissues: It depends on the manufacture of nanomaterials that work to improve the bioavailability of the drug (Bioavailability) This means the presence of drug molecules in the target place of the body 7 Nanotechnology can offer drug delivery solutions in the following areas:

a- Drug Coating: Using pharmaceutical packaging materials such as liposomes and polymers (such as polylactide –PLA, and lactide combined with PLGA glycolide)

b- Drug carriers:Another type of drug delivery system, nanotechnology offers important, manageable solutions to link with the drug, the target and the imaging material, 18
Research Approach

The researchers adopted the descriptive analytical approach for the analysis of subjects in the Department of Biology in the Colleges of Education.

Search procedures

First: Research Community (Community of Subjects)

The community of subjects covered represents all subjects for the four stages of the Biology department in the Colleges of education.

Second: Research sample (sample content of subjects): The analysis process included the content of subjects in the Department of Biology in the College of Education, as the number of analyzed subjects (15) of the 35 articles, or 43% of the total community. The total analyzed pages are (12201) pages, (1689) pages of the first stage materials, (2291) pages of the second stage materials, (4367) pages of the third stage materials and (3854) pages of the fourth stage materials. The scientific, drawings, tables and charts after the indexes and glossary of terms for the analyzed materials were excluded.

Analysis of the content of the subjects of the Department of Biology

The researchers relied on the explicit idea and the implicit idea as the two recording units because the subjects in the Department of Biology are scientific materials and expressions are often clear and explicit, but their use of the idea implicit, because some topics can suggest ideas that reflect the issues or sub-issues of the standard nanotechnology.

Validity of the analysis

To ensure the validity of the analysis conducted by the researchers were hired two experts * in the methods of teaching Biology and presented to them three subjects (cytology, biology, algae) with the standard of nanotechnology issues that were built and ideas extracted from the content of analyzes to make sure The analysis was valid, and they unanimously agreed on the validity of the analysis, which was prepared by the researchers in truth to the analysis he conducted.

Stability of analysis

In order to be objective analysis and to obtain acceptable stability, the researchers used two types of stability:

1- Agreement over time

To calculate the stability coefficient in this way, the researchers re-analysis after thirty days as the value of the coefficient of stability calculated (0.98) using the Holste equation, a very high value and reflect a high degree of stability.

2- Agreement between analysts

The researchers used external analysts to analyze the content *. This was done by selecting a random sample of the total analyzed content of (12201) pages. The proportion of the stability sample represented about (20%) (2790 pages), which included books (cell biology, biology, algae) and using the equation (Holst) was extracted the stability coefficients calculated in this way and was equal to (0.82) For the researcher with the first analyst and (0.80) For the researcher with the second analyst (0.83) between the first and second analyst, and thus the coefficient of stability is good, and some studies have indicated that the acceptable stability ranges between (0.50 or 0, 60) and above 12 as in Table 1

<table>
<thead>
<tr>
<th>Agreement through time</th>
<th>Researchers after 30 days</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Agreement between analysts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between the researchers and the first analyst</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td>Between the researchers and the second analyst</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>Between the first and second analyst</td>
<td>0.83</td>
<td></td>
</tr>
</tbody>
</table>

Determine the spoken ratio to compare the results of the analysis
Depend that the percentage (20%) to be a hypothetical ratio to compare the results of the analysis based on the agreement of the arbitrators and experts on this ratio, the researchers presented a questionnaire to find out the ratio (2). The agreement was (80%) of the experts and arbitrators on this ratio.

The results will be presented according to the research objective and discussed as follows:

Identify the extent to which the content of subjects is included in the Department of Biology, College of Education for Nanotechnology Issues.

After the subjects were subjected to the four stages, the pages subjected to analysis included (12201) pages and the results were as shown in Table (2).

**Table (2) Frequencies and Percentages of Nanotechnology Issues in the Subjects of Biology Departments**

<table>
<thead>
<tr>
<th>No</th>
<th>Subjects</th>
<th>Total iterations of major and minor issues</th>
<th>Percentage of Origin (16) element of a case sub-extracted</th>
<th>Order</th>
<th>percentage Realized% of Origin (90) element Issue by standard</th>
<th>percentage Realized from all the subjects Tuition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Materials of the first stage</td>
<td>4</td>
<td>25%</td>
<td>second</td>
<td>4.44%</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Materials of the second stage</td>
<td>1</td>
<td>6.25%</td>
<td>Third</td>
<td>1.11%</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Materials of the third stage</td>
<td>11</td>
<td>68.75%</td>
<td>First</td>
<td>12.22%</td>
<td>17.77%</td>
</tr>
<tr>
<td>4</td>
<td>Materials of the fourth stage</td>
<td>0</td>
<td>0%</td>
<td>Fourth</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>16</td>
<td>100%</td>
<td></td>
<td></td>
<td>17.77%</td>
</tr>
</tbody>
</table>

From the data recorded in Table (5) it is clear that the subjects in the third stage were the most interested in the contents and issues of nanotechnology, where it obtained 68.75% of the percentage out of (16) elements of the sub-issue extracted, followed by the first stage by 25% and the second by 6.25% and then the fourth by (0%) and the researchers attribute this to the algae materials and practical environment and educational laboratory. In the third stage, it received the most attention, followed by the first stage, which was the theoretical and practical biology and theoretical cell which focused on the issues of nanotechnology and microscopes. Then the second stage materials represented theoretical histology material while the fourth stage materials were weaker and poorest as it did not get any repetition despite the importance of the fourth stage materials in the formation of specialization for students of the Department of Biology in the colleges of education and this is a take on the materials of that stage, which was expected to the strongest of the four stages because the first stage represents the beginning of specialization and the second and third stages represent a transitional stage to the end of the specialization of the fourth stage.

As for the issues, the diversity of interest in the main and sub-issues and their components, as in Table (3).
Table 3: Frequencies and percentages of paragraphs achieved from the Nanotechnology Standard

<table>
<thead>
<tr>
<th>No</th>
<th>The main issues</th>
<th>Repeat paragraphs</th>
<th>Percentages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Introductory issues of nanotechnology</td>
<td>11</td>
<td>68.75%</td>
</tr>
<tr>
<td>2</td>
<td>Agricultural issues</td>
<td>1</td>
<td>6.25%</td>
</tr>
<tr>
<td>3</td>
<td>Environmental issues</td>
<td>2</td>
<td>12.5%</td>
</tr>
<tr>
<td>4</td>
<td>Issues related to the dangers of nanotechnology</td>
<td>1</td>
<td>6.25%</td>
</tr>
<tr>
<td>5</td>
<td>Future issues</td>
<td>1</td>
<td>6.25%</td>
</tr>
<tr>
<td>6</td>
<td>Medical and health issues</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>Industrial and economic issues</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>Food issues</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>16</td>
<td>100%</td>
</tr>
</tbody>
</table>

Through Table (3) we note that the issues in general did not get sufficient attention in the subjects of the four stages, which indicates the weakness of keeping up with these materials to the developments of science and its applications, including nanotechnology, but when looking at the same table we note that the identification issues of nanotechnology have been obtained more interest of the subjects has obtained (11) repetitions out of (16) recorded for all subjects and the rate of (68.75%) and the researchers attribute this to the fact that most of the study material deals with these tariff issues as an introduction to nanotechnology as well as the standards and microscopes that adopt this technique.

Conclusions

Through the results of the research, the researchers reached the following conclusions: The subjects of the Department of Biology in the colleges of education were weak in their inclusion of nanotechnology issues. The subjects of the third stage in the Department of Biology got the highest frequency, followed by the first stage and then the second stage, while the fourth stage did not receive any repetition. The nanotechnology knowledge issues with the greatest interest in the content of the subjects were the four stages.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Al-Qadissiyah, College of Education, Iraq and all experiments were carried out in accordance with approved guidelines.

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Color Stability of Different Aesthetic Resin Composite Materials: A Digital Image Analysis

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Abstract

This study aimed to evaluate and compare the color stability of three commercially available resins composites after exposure to different staining solutions using digital image analysis. A total of ninety-disc shape specimens of a microhybrid composite (Amelogen plus), ultrafine hybrid composite (Essentia), and nanohybrid composite (Beautifil II) were produced in Teflon mould (n=30/resin composite type). Specimens of each resin composite were then divided into 5 subgroups (n=6/subgroup) and immersed in 200 ml of Pepsi, orange juice, tea, coffee and artificial saliva (control group), respectively. Digital images of the specimens were taken before (baseline) and after 28 days immersion against a black and white background. Adobe Photoshop CS6, Ver.13.0.1 graphic program (AdobeSystems Inc., San Jose, CA, USA) was used to analyze the digital images and calculate the change in color (ΔE). Data were submitted to statistical analysis using one-way ANOVA and Tukey Posthoc test at a significance level of p<0.05. This study concluded that all resin composites exhibited color changes after immersion in all staining solutions. However, Amelogene Plus showed better resistance to staining compared to Beautifil II and Essentia.

Keywords: aesthetic resin, composite materials, image analysis

Introduction

Composite restorative materials are the most popular esthetic materials used in dental practice. The color instability of composite materials may be the main reason for the replacement of restorations. Discoloration of composite resin caused by extrinsic or intrinsic factor. Intrinsic factors include physical-chemical discoloration reactions in the composite matrix, in surface and deeper layers of the material, triggered by heat, humidity, or UV irradiation. Chemical discoloration has been attributed to a change or oxidation in the amine accelerator, oxidation in the structure of the polymer matrix, and oxidation of the unreacted pendant methacrylate groups ¹. Extrinsic factors are related to the surface absorption of staining agents from exogenous sources or due to the accumulation of plaque ², or superficial degradation of the restorative materials and their adsorption of staining agents ³. The degree of color change can be influenced by various factors such as incomplete polymerization, chemical reactivity, water sorption, oral hygiene, diet, and surface smoothness of the restoration⁴. The composition of the composite resin and the characteristics of the particles have a direct effect on the surface smoothness and susceptibility to extrinsic staining ⁵. Consumption of certain drinks such as Tea and coffee may affect the physical properties and aesthetic of composite restoration. The effect of drinks on the properties of restorative resins may be directly related to the amount and frequency of its intake ⁶. The aim of this study was to evaluate and compare the color stability of three commercially available resins composite: Amelogen Plus (Microhybrid), Essentia (ultrafine hybrid), Beautiful II (Nanohybrid) after exposure to different staining solutions for 28 days using digital image analysis. The null hypothesis tested was that there is no difference in color changes (ΔE) between the tested composite materials after 4 weeks of immersion in different staining solutions.

Materials and Method

One microhybrid composite (Amelogen plus, Ultradent), one ultrafine hybrid composite (Essentia, Ultradent), one nanohybrid composite (Beautifil II, GC America).
GC), and one nanohybrid composite (Beautifil II, Shufo) were evaluated in this study. For each brand, the enamel shade was selected. A total of ninety-disc shape specimens of composite materials were produced in Teflon mould (10 mm diameter x 1 mm thick). After filling the mould with the composite, the discs were covered with glass slides, to exclude atmospheric oxygen, and then cured by visible light for 40 s, using a dental curing unit (Optilux, Demetron Res Crop, Danbury, USA) with an irradiance of 850 mW.cm⁻², which monitored with a radiometer (Kerr/Demetron, Danbury, USA). Light-curing was repeated on the opposite surface for another 40 s. After which, specimens were stored in an incubator at 37°C for 24 hours. Specimens of each resin composite brand were then divided into 5 subgroups (n=6/subgroup) according to the immersion solutions represented by Pepsi, orange juice, tea, and coffee or artificial saliva which constituted the control group. The digital images acquisition of all specimen groups was taken before immersion (baseline) by digital imaging technique using an SLR camera (Nikon D5200, Nikon Corporation, Japan) with a 105mm camera macro lens (Sigma 105 EX Macro, Sigma, Japan). The camera was fixed perpendicularly on stand clamp holder (10 cm distance from the specimen) and set on manual mode, which allowed total control of shutter speed 1/6, ISO 1250, f-stop 3.5. These measurements were remained unchanged during taking shots and three images were obtained for each sample against a white as well as a black background. For the digital imaging method, four fluorescent tubes were mounted on a costume made photostand with tubes perpendicular to the front plane that hold the discs, being 20 cm away from the specimen and illuminating at an angle of 45°. Two 6,500-K fluorescent tubes (Philips PL-C 18W/865, Koninklijke Philips Electronics N.V., Eindhoven, Netherlands) were placed in the lower sockets and were combined with two 2,700 K (Philips PL-C 18W/827) fluorescent tubes placed in the upper sockets. After baseline images acquisition were made, specimens were then immersed in 200 ml of each staining solution and kept in an incubator at 37°C for 28 days. Staining solutions were changed every week to avoid bacteria or yeast contamination. After the staining period, the specimens were gently rinsed with distilled water and air-dried. Imaging measurements were repeated for each sample to determine the color variation.

The digital images were transferred to a personal computer (PC), saved as TIFF images and were analyzed using the Adobe Photoshop CS6, Ver.13.0.1 graphic program (AdobeSystems Inc., San Jose, CA, USA). For standardized calculations, a measurement template was created in the middle third of the samples that consisted of a square area of 50 pixels. The CIE L*a*b* values of these particular areas were calculated using the eyedropper tool. The color data obtained directly in color picker palette tab for (L, a and b) parameters (Figure1). Color changes (ΔE) were calculated as follows:

\[
\Delta E = \sqrt{(L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2}
\]

where L* is lightness (0 = black; 100 = white), a* is green-red component (−a* = green; +a* = red), b* is blue-yellow component (−b* = blue; +b* = yellow), subscript 1 is the baseline measurement before the immersion and subscript 2 is the measurement after 28 days immersion in staining solution.

Figure 1: Adobe photoshop program shows how to pick the color data from the sample (L=47, a=−8, b=10) red dotted area.
Data of color change acquired from black, white backgrounds were submitted to statistical analysis using one-way ANOVA and Tukey Posthoc test for multiple comparisons between groups at a significance level of p<0.05. Statistical analysis performed using the Statistical Package for the Social Sciences (Version 24; SPSS Inc., IBM, Chicago, Illinois, USA).

**Results**

Tables 1 and 2 show ΔE±(SD) of all tested composite resins after 28 days immersion in different staining solutions on a black and white background, respectively. The lowest ΔE value observed for Amelogen Plus composite resin in artificial saliva on a black background (1.42±(0.45)), on the other hand, the highest ΔE value observed for Essentia composite resin in coffee on white background (28.0±(1.12)). Regarding staining solutions, coffee caused the highest ΔE in all composite resins, followed by tea, orange, Pepsi and artificial saliva, as shown in tables 1 and 2.

In the black background data set (Table 1), there were significant differences in ΔE in each brand of resin composite between the different staining solutions (p<0.05), except between the artificial saliva and Pepsi, there was no significant difference in ΔE in all brands of resin composites (p>0.05). Also, there were significant differences (p<0.05) in ΔE between the three composite resins in each staining solution except between Amelogen Plus and Beautiful II in artificial saliva, Pepsi, orange and tea (p>0.05), there was no significant difference in ΔE. Similarly, there was no significant difference in ΔE between Essentia and Beautiful II composite resins in orange staining solution (p>0.05), as shown in table 1.

**Table 1:** Change in color ΔE ± (SD) measured on the black background of all tested composite after 28 days immersion in different staining solutions.

<table>
<thead>
<tr>
<th>Black background</th>
<th>Artificial Saliva</th>
<th>Pepsi</th>
<th>Orange</th>
<th>Tea</th>
<th>Coffee</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amelogen Plus</td>
<td>1.42±(0.45)</td>
<td>2.53±(0.58)</td>
<td>4.41±(0.95)</td>
<td>8.66±(0.81)</td>
<td>11.72±(0.56)</td>
</tr>
<tr>
<td>Essentia</td>
<td>2.87±(0.29)</td>
<td>3.69±(0.46)</td>
<td>5.52±(0.73)</td>
<td>12.09±(1.08)</td>
<td>22.23±(1.06)</td>
</tr>
<tr>
<td>Beautiful II</td>
<td>1.76±(0.30)</td>
<td>2.18±(0.83)</td>
<td>5.14±(0.16)</td>
<td>9.82±(0.87)</td>
<td>13.39±(0.93)</td>
</tr>
</tbody>
</table>

Superscript small letters represent non-significant difference between relevant groups.

In the white background data set (Table 2), there were significant differences in ΔE in each brand of resin composite between the different staining solutions (p<0.05) except in Amelogen Plus and Beautiful II there was no significant difference in ΔE between the artificial saliva and Pepsi (p>0.05). Also, there were significant differences (p<0.05) in ΔE between the three composite resins in each staining solution. However, there was no significant difference in ΔE between Amelogen Plus and Beautiful II in coffee solution (p>0.05), as shown in table 2.

**Table 2:** Change in color ΔE ± (SD) measured on the white background of all tested composite after 28 days immersion in different staining solutions.

<table>
<thead>
<tr>
<th>White background</th>
<th>Artificial Saliva</th>
<th>Pepsi</th>
<th>Orange</th>
<th>Tea</th>
<th>Coffee</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amelogen Plus</td>
<td>1.8±(0.58)</td>
<td>2.8±(0.68)</td>
<td>5.9±(0.6)</td>
<td>11.85±(0.51)</td>
<td>18.0±(0.93)</td>
</tr>
<tr>
<td>Essentia</td>
<td>5.18±(0.52)</td>
<td>7.85±(0.47)</td>
<td>12.03±(0.32)</td>
<td>18.22±(0.79)</td>
<td>28.0±(1.12)</td>
</tr>
<tr>
<td>Beautiful II</td>
<td>3.01±(0.73)</td>
<td>4.07±(0.82)</td>
<td>7.92±(0.57)</td>
<td>14.75±(1.42)</td>
<td>19.64±(1.99)</td>
</tr>
</tbody>
</table>

Superscript small letters represent non-significant difference between relevant groups.
Discussion

As a composite restorative material are continuously exposed to saliva, food stains and beverage pigments, it is important to determine its susceptibility to color change. In the present study, we investigated the effects of beverages tea, coffee, Pepsi and orange juice on the color stability of different composite materials. The artificial saliva constituted the control group. The results of the present study showed that there is a difference in color changes ΔE between the tested resin composites after immersion in different staining agents.

For standardization of staining conditions, we perform an “in vitro” study to control all variables such as food or drinking habits, tooth brushing. All the tested composites were of enamel translucency A2 shade, enamel translucency was selected because it is used as the most external layer and being in contact with staining agents. The immersion time for each composite group was 28 days in accordance with previous studies. Ardu et al. in 2017 reported that the immersion time for 28 days simulated about 2 years of drink consumption. Ertas et al. considered 28 days is equivalent to about 2.5 years of clinical aging (24 h in vitro staining corresponds to 1 month in vivo).

Digital imaging system is a reliable method for determining the color of teeth and gingiva when used with the appropriate calibration protocols. In this study, the digital imaging system was selected to evaluate the color changes against white and black background which were used to allow a double evaluation to simulate two different clinical situations; black background can mimic the situation, where no tooth structure exists in the back. i.e., class IV composite filling. White background can mimic class I, II, III, V and veneers, where one of the walls is still present.

Results of this study revealed that all tested composite showed significant color changes after immersion in the tested solution. Amelogen Plus was more stain-resistant followed by Beautiful II, while Essentia seems to be the most prone to staining. It seems that Essentia extensively colored in these staining solutions. The slightly different ranking obtained with the two different backgrounds may be related to different opacity of the composites which might modify the color perception on different backgrounds. The general behavior trend of the tested composites is confirming that Essentia has less color stability than Amelogene Plus and Beautiful II.

Findings of this study may depend on the hydrophilicity of the resin matrixes of these three resin composites and their filler particles. The differences in the staining resistance between these resin composites may be attributed to the different filler size and load which may affect the overall resin content in the cured composite. Essentia contains prepolymerized filler of resin, which together with the resin matrix, may increase the resin content in the composite, hence the water sorption and staining susceptibility consequently. It has been reported that increased resin content in the composite may increase the water sorption significantly especially in the first week of immersion in the staining solution.

The structure of resin matrix and filler type may have a direct impact on the susceptibility to extrinsic staining. Changes in ΔE value were greater for the tested staining solutions than for the control group, so the staining effect of a staining solution varied according to its ingredients. These results are in agreement with many previous studies which shown various staining potential of these drinks and this may be related to their composition and properties.

Regarding the staining solution tested, coffee showed the most staining capacity, followed by tea, orange, Pepsi and artificial saliva (Control group). The highest values of ΔE reached was in coffee and this could be related to its acidity (pH 4.5), both adsorption and sub-surface absorption of coffee colorant. The degree of polarity of the staining agent determines their degree of penetration into the resin materials. Less degree of polarity, such as coffee, can easily penetrate into the polymer matrix. Both coffee and tea contain a large amount of staining agents like Gallic acid, which could be another reason for the staining capacity of these materials. This finding is in agreement with the study conducted by Ardu et al. Some studies reported that a solution with a lower pH can attack the composite surface, causing some changes and increasing pigment absorption. Although Pepsi had a low pH, which can damage the surface integrity of the materials, it did not
cause as much staining as coffee and tea. This may due to the absence of yellow colorant, which is abundant in coffee and tea\(^1\), these findings were in agreement with other studies which found that coffee and tea produced more discoloration than cola\(^{17,18}\).

**Conclusion**

This study concluded that staining solutions affected the color stability of the tested resin composites significantly in a clinically unacceptable level, except for Amelogene Plus and Beautiful II in artificial saliva and Pepsi, which were acceptable clinically. Also, Essentia resin composite performed inferior to Amelogene Plus and Beautiful II in terms of color stability.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of Dentistry, University of Baghdad, Baghdad, Iraq and all experiments were carried out in accordance with approved guidelines.

**References**

Bergamot Essential Oil Effect against Candida Albicans Activity on Heat Cure Acrylic Denture Base

Zinah Salah Mawlood1, Ghassan Abdul-Hamid Naji2

1 M.Sc. Student, 2 Ass. Prof. (Department of Prosthodontics, College of Dentistry/ University of Baghdad, Iraq

Abstract

The aim of the current study is to evaluate the antifungal efficacy of several different percentages of Bergamot Essential Oil (BEO) incorporated into heat cure PMMA denture base material against Candida albicans.

Materials and Method: Eighty samples were divided into eight groups including six various percentages of BEO (2%, 3%, 4%, 5%, 6% and 7% by volume) and 0% BEO as a control group, in addition to 1.4% by weight of nystatin which represent the positive control group. All these additives were incorporated into heat cure PMMA denture material. After 48h incubation in distilled water, all samples were assessed by Candida albicans colonies viable count test.

Results: For Candida albicans activity test; the experimental groups (2%, 3%, 4%, 5%, 6% and 7% of BEO) showed highly significant decrease in the mean values of the viable count of Candida albicans when compared to the control group (0% BEO) (p<0.01). In contrast, a non-significant difference among experimental groups and 1.4% nystatin group.

Conclusion: Bergamot essential oil was successfully incorporated into heat cure PMMA denture base material and could act as potential antifungal agent with a drug delivery system against Candida albicans. It seemed that adding of 5% and 6% BEO was the most beneficial effects against the growth of fungi.

Key words: Heat cure PMMA, Denture base, Bergamot essential oil, Nystatin, Candida albicans

Introduction

Walter Wright discovered poly(methyl methacrylate) (PMMA) after publishing the results of his clinical evaluation of PMMA in 1937, since it is introduced has become most superior, popular and satisfactory of all polymeric denture base materials. Acrylic denture base is used in removable dentures fabrication, and its popularity and universal use has attributed to low cost, light weight, ease of processing, ease of reparability, low water sorption and solubility, biocompatibility, satisfactory aesthetic properties and accurate fit, in spite of that this material until now remain not ideal denture base material due to the presence of several drawbacks like susceptibility to microbial colonization and formation of biofilm on its surface and this make the denture a source of different infections because it harbor the microorganisms in addition to lack of manual dexterity among the old age patients which make the effective biofilm removal not possible. Denture stomatitis is a disease related to denture use, and considered chronic atrophic oral candidiasis, which affect up to 65% of denture wearer, its etiology is multifactorial, but Candida albicans considered the main pathological microorganism which is the mostly isolated type from the oral cavity in patient with denture stomatitis. The most common line that is used in treatment of denture stomatitis is prescribing of topical antifungal medicines, but maintaining optimal oral drug dose and lacking of motor dexterity of geriatric patient who had impaired cognition, limit their use and make it challenging to get a maximum benefit of these topical drugs. To overcome these obstacles it is
better to incorporate the antifungal drugs into denture base materials. Unfortunately fungal resistance and side effects of these drugs make it necessary to obtain naturally derived medicaments as a substitution to these synthetic drugs. Herbal medicines are a powerful alternative treatment for microbial infections in the oral cavity which have less or no side effects; and this made a worldwide trend to make a lot of researches about them in order to find biologically safe herbal-based medicines with effective antifungal properties. Plants oils are herbal medicines and various researches, recently, have been done to test their antifungal efficiency against Candida albicans and they reported that these oils are considered a promising line of therapy which have effective antifungal properties and can be used for treatment of denture stomatitis. One of the recent research was done through the incorporation of virgin coconut oil into heat cured acrylic-based denture soft lining and proved the antifungal efficiency of this oil against Candida albicans. Bergamot is the popular name for Citrus bergamia Risso et Poiteau, Citrus bergamia is defined as “a hybrid between a sour orange (C. aurantium L.) and lemon (C. limon L. Burm.F.) or a mutation of the latter. Other authors considered it a hybrid between a sour orange and lime”.

Materials and Method

Regular- conventional heat cure acrylic resin denture base material (Vertex, Netherlands) was utilized. Bergamot essential oil (bergaptene-free) from (Aura Cacia pure essential oils, USA) was added to the liquid part of acrylic resin in six different percentages (2%, 3%, 4%, 5%, 6% and 7%), these percentages were selected to discover which one is as effective as 1.4% nystatin group (positive control group). This proportion of nystatin was calculated by the conversion of International Units (U) to milligrams. Every 6079U corresponded to 1 mg of nystatin. Therefore, for this group (500,000U) the amount of nystatin used was 90 mg for every 6.6 g of acrylic powder. In general, 500 000 units was the most common concentration used by many authors and result in decreased yeast count compared to control group. Also this percentage of nystatin start to change the color of acrylic resin into orange so it is objectionable to use it above this percentage especially in aesthetic area.

Study design

Total of eighty samples were prepared, 10 samples for control group, 10 samples for each percentage of BEO groups (2%, 3%, 4%, 5%, 6% and 7% BEO) and 10 samples for positive control group. All samples were tested by viable count of Candida albicans colonies test at the same time and circumstances and they stored in distilled water for 48h prior to test procedure to reach the state of standardization.

Candida albicans colonies viable count test

Samples preparation

Plastic models were prepared with dimensions of 10×10×2.3 mm in length, width and thickness, respectively using laser cutting machine. Then, the models were invested in freshly mixed type IV extra hard dental die stone to create stone molds where the acrylic resin samples will be packed. For control group the acrylic resin denture base materials were proportionate and mixed as directed by the manufacturer instructions. Regarding BEO incorporated samples, the required amount of oil were measured by micropipette and subtracted from the volume of monomer, then mixed manually in dry clean glass beaker with monomer. Following which, the mixture was added to acrylic powder and mixed thoroughly.

Isolation and identification of Candida albicans

Candida albicans were obtained from oral cavity of patient with an indication of a denture induced stomatitis by using cotton swab which then cultured on the surface of a sabouraud dextrose agar SDA (Oxoid, United kingdom) plates and incubated at 37°C for 48 h. The identification of candida albicans was done according to the following order: Firstly, the colony morphology (macroscopic examination) in which the Candida albicans appear as creamy, pasty, smooth and convex colonies on SDA. Secondly, microscopic examination using Grams stain procedure , candida seen as small gram-positive oval or budding yeast cells. Thirdly, Germ tube formation. Fourthly, Biochemical identification using analytical profile index API Candida system (bioMérieux, France) and API 20 C AUX system.

Assessment of Candida albicans viable count

Candida albicans suspension of about 10^7CFU/mL which equal to 0.5 McFarland standards was prepared by diluting a small quantity of inoculums in a test tube containing normal saline and measuring this solution with McFarland densitometer device as shown in Figure 1(A). Then, 100μL of Candida suspension was added
to each tube contain 9.9 mL of sabouraud dextrose broth (Oxoid, England) to obtain 10 mL broth mixture this done by using micropipette. The samples after that immersed in tubes (Figure 1 (B)) and incubated at 37 °C for 24 h. After incubation, about 100µL was taken from all tubes of broth mixture and added to other tube contain 9.9 mL normal saline so tenfold dilution obtained. A second dilution made from this dilution by the same procedure as in Figure 1(C). About 100 µL taken from the second dilution and spreaded on SDA plates by using disposable inoculation loops (Figure 1 (D)). The SDA plates incubated aerobically at 37 ºC for 24 h. This dilution was chosen because it demonstrated a countable range of 30-300 CFU.

Following incubation, the Candida albicans colonies were visible on SDA plates, all these colonies counted and this viable count analyzed statistically so the material antifungal efficacy (AFE) was calculated using following formula (Equation 1):

$$AFE [%] = \frac{V_c - V_t}{V_c} \times 100 \%$$

In this formula the number of viable colonies of control samples was represented by $V_c$ and number of viable colonies of experimental samples was represented by $V_t$

**Statistical analysis**

The data of this research were collected and analyzed using SPSS (statistical package for social science – version 21) computer software. Descriptive statistics were made which include Means, Standard deviation, Minimum, Maximum. Inferential statistics include ANOVA (one-way ANALYSIS OF VARIANCE) test which was used to evaluate the significance of difference among the mean values of all groups, then multiple comparison using Dunnett t-tests which treat one group as a control, and compare all other groups against it was used.
Results

The viable count of Candida albicans colonies test was performed on six experimental groups (2%, 3%, 4%, 5%, 6% and 7% BEO) and two control groups (negative control 0% BEO group and positive control 1.4% nystatin group for comparison). After 48 h of samples incubation in distilled water, the results of viable counts of Candida albicans showed a reduction in the mean values for experimental groups in comparison to negative control group. So 5% and 6% groups showed the lowest mean value among all groups, while other experimental groups (2%, 3%, 4% and 7%) revealed higher mean values than positive control group. Antifungal efficiency (AFE) for the experimental groups are as follows: 55.53% for 2% BEO group, 67.21% for 3% BEO group, 68.64% for 4% BEO group, 76.43% for 5% BEO group, 74.18% for 6% BEO group, 66.80% for 7% BEO group and 69.87% for 1.4% nystatin group.

Descriptive statistics (mean, standard deviation, maximum and minimum) and statistical test of viable count of C. albicans results using One-Way ANOVA for comparison of means of all studied groups listed in Table (1) the difference between groups was highly significant. For multiple comparison purpose the Dunnett t (2-sided) a test was used. In this test the 1.4% nystatin group taken as control group and all other groups compared against it. The 0% BEO group showed a highly significant difference while all other experimental groups showed a non-significant difference in comparison to 1.4% nystatin group as listed in Table (2).

Table (1): Descriptive statistics of viable count of Candida albicans, using One-Way ANOVA

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean (CFU/mL)</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>162.667</td>
<td>18.877</td>
<td>142.000</td>
<td>179.000</td>
<td>27.245</td>
<td>0.000</td>
</tr>
<tr>
<td>2%</td>
<td>72.333</td>
<td>10.116</td>
<td>66.000</td>
<td>84.000</td>
<td></td>
<td>HS</td>
</tr>
<tr>
<td>3%</td>
<td>53.333</td>
<td>15.631</td>
<td>39.000</td>
<td>70.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4%</td>
<td>51.000</td>
<td>11.000</td>
<td>40.000</td>
<td>62.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5%</td>
<td>38.333</td>
<td>13.051</td>
<td>28.000</td>
<td>53.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6%</td>
<td>42.000</td>
<td>17.692</td>
<td>26.000</td>
<td>61.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7%</td>
<td>54.000</td>
<td>11.136</td>
<td>42.000</td>
<td>64.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.4% Nystatin</td>
<td>49.000</td>
<td>4.583</td>
<td>44.000</td>
<td>53.000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (2): Multiple comparisons test of Candida albicans (CFU/mL) among the groups using Dunnett t (2-sided)a test

<table>
<thead>
<tr>
<th>Multiple Comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dependent Variable: Canalb</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Dunnett t (2-sided)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I) groups</td>
</tr>
<tr>
<td>control</td>
</tr>
</tbody>
</table>
Cont... Table (2): Multiple comparisons test of Candida albicans (CFU/mL) among the groups using Dunnett t (2-sided)* test

<table>
<thead>
<tr>
<th>Group (%)</th>
<th>Nystatin (mg/mL)</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>2%</td>
<td>1.4%</td>
<td>23.33333</td>
<td>.214</td>
</tr>
<tr>
<td>3%</td>
<td>1.4%</td>
<td>4.33333</td>
<td>.999</td>
</tr>
<tr>
<td>4%</td>
<td>1.4%</td>
<td>2.00000</td>
<td>1.000</td>
</tr>
<tr>
<td>5%</td>
<td>1.4%</td>
<td>-10.66667</td>
<td>.867</td>
</tr>
<tr>
<td>6%</td>
<td>1.4%</td>
<td>-7.00000</td>
<td>.980</td>
</tr>
<tr>
<td>7%</td>
<td>1.4%</td>
<td>5.00000</td>
<td>.997</td>
</tr>
</tbody>
</table>

*The mean difference is significant at the 0.05 level.

Figure 2: Viable counts of Candida albicans after 48 hours incubation of: a) Control samples; b) some experimental samples with BEO; c) positive control sample (1.4% nystatin). Medicinal Plants extracts are very good replacement to antimicrobial drugs with less or without side effects, as a result this encouraged the worldwide tendency towards herbal-based medicines and a lot of researches were done to obtain herbal medicine that is biologically safe and have excellent antifungal properties. Essential oils are examples of naturally derived herbal medicaments which are concentrated, hydrophobic liquids extracted from plant and have wide spectrum of pharmacological activities, these essential oils considered as a promising therapeutic line for oral infections and in the last few decades a lot of kinds of oils have been tested for efficacy against Candida albicans, each one of them has special active component that affect the fungi in particular mechanism. Bergamot essential oil has been used for its antiseptic, anti-inflammatory, diaphoretic, appetizing, and analgesic effect.

Conclusion

Within the limitations of the present study; the following conclusions can be obtained that Bergamot essential oil was incorporated successfully into heat cure acrylic resin denture base material and worked as a powerful antifungal herbal medicament against Candida albicans that is comparable with the effect of nystatin. Moreover, the samples with 5% and 6% BEO revealed a better antifungal efficiency compared to all other control and experimental groups.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Dentistry/University of Baghdad, Iraq and all experiments were carried out in accordance with approved guidelines.

References

3. Rasmy AHM. Effect of microwave cured acrylic resin on candidal growth in complete denture. 2009;


Antimicrobial Activity and Characterization of Some Oxazole, Thiazol and Quinoline

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1Department of Chemistry, College of Science-University of AL-Mustansiriyah- Baghdad-Iraq

Abstract

New Heterocyclic compounds derivatives comprising 1,3-oxazole, chalcone,thiazole, pyrimidine, quinolone moieties are reported. New derivatives of Quinazolin-4 (3H)-one ring comprising Schiff’s bases,(1,3,4-Thiadiazole),(1,3,4-Oxadiazole) and (1,2,4-Triazole), Thiaurease moieties are reported. Compounds (1), (2) and (5) were synthesized by reaction of benzoyl chloride with urea , thiourea and anthranilic acid respectively , then compounds (1)and (2) were converted into(3a-c) and (4a-c) derivatives. While compound (5) reaction with urea to convert to compound (6) which was converted to (7a-c) . chalcone derivatives (9a,b ) were readily obtained by reaction of compound (8) with different aldehydes, Compounds (9a, b) were converted into (10a,b) and (11a,b) The structure of these compounds has been established on the basis of their spectral data FTIR and 1H NMR. These compounds were tested for invitro antibacterial activity against Escherichia coli, Sepidermidis, S.aureusand Klebesillastandard methods. These synthesized compounds have been shown moderate to good antibacterial activity.

Keywords: 1,3 oxazole, chalcone, thiazole, pyrimidine, quinolone, antimicrobial activity.

Introduction

Heterocyclic compounds had been receiving considerable attention due to their pharmacological and pesticidal importance1 . The heterocyclic nitrogen compounds like quinazolinone derivatives has a vital role in synthetic drugs and biological processes. A Quinazolin-4-one derivative possessing broad spectrum of biological and pharmacological activities such as antifungal 2, antimicrobial 3, bronchodilator 4,antihistaminic 5, anti-inflammatory 6,1,3-Oxazole and thiazole derivatives possess a broad spectrum ofpharmacological activities such as antibacterial 7-9, antiviral, anti-inflammatory10,11, antitumor 12. The biological significance of the pyrimidine derivatives has led to the synthesis of substituted pyrimidine and their derivatives13, pyrimidine derivatives have been important role in medical applications. One possible reason for their activity is the presence of a pyrimidine base in cytosine, thymine and uracil which are essential building of nucleic acids, RNA and DNA. Pyrimidine derivatives possess several interesting biological activities such as antimicrobial 14-15, antitumor 16, antifungal 17, antimalarial, 18, anticancer 19, antiallergic 20, antitubercular activities 21 and anti-inflammatory activities 22. Many Pyrimidinederivatives are used for thyroid drugs and leukemia 13.

Materials and Method

Apparatus

Melting point were determined in open capillary tubes and were uncorrected and the purity of the compounds were checked by TLC. The IR Spectra were recorded on a Perkin-Elmer 1600 series FTIR spectrometer, using KBr discs. 1H NMR Spectra of prepared derivatives were recorded in DMSO with TMS as internal standard on aVarian-Mercury 300 MHz Spectrometer the reaction were followed.

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A mixture of benzoyl chloride (1.1 mole), and urea (1.1 mole) in dry benzene (30 ml) were mixed in round bottom flask and refluxed for 15 hrs, resulting mixture was poured in to crush ice and the solid filtered and recrystallized from appropriate solvent table 1.

Synthesis of N-(aminocarbonothioyl)-benzamide (2)

A mixture of thiourea (1 mole) and (1 mole) benzoyl chloride were dissolved in benzene (30 ml) then refluxed for (16 hrs.), the mixture was cold and filtered to obtained the solid, and purified by recrystallization table 1.

Synthesis of N-substituted acetamide (General method)

In adry and clean conical flask (0.01 mole) of substituted aromatic amine was dissolved in 15 ml dry benzene with continuous shaking and (0.015 mole) of chloro acetyl chloride was added drop wise by dropping funnel, the mixture was heated in water bath for (1 hr) after the reaction complete, the product which separated were filtered and washed with sodium bicarbonate (5%) , water, dried and recrystallized from alcohol.

Procedure for Synthesis of derivatives (3a-c)

A mixture of compound (1) (0.01 mole) and (0.01 mole) of 2-chloro-N- (substituted phenyl)acetamide in ethanol were refluxed for 24 hrs and allowed to stand undisturbed over night, the product separated on cooling was filtered and purified by recrystallization.

General Procedure for Synthesis of derivatives (4a-c)

A mixture of compound (2) (0.01 mole) and (0.01 mole) of 2-chloro-N- (substituted phenyl)acetamide in ethanol were refluxed for 24 hrs and allowed to stand undisturbed over night, the product separated on cooling was filtered and recrystallized from appropriate solvent table 1.

Synthesis of (2-phenyl)-1,3-benzoxazin-4(H)ones (5)

A mixture of benzoyl chloride (1.4 gm, 0.01 mole) and anthranilic acid (1.38 gm, 0.01 mole) in triethyl amine (30 ml) were stirred at 0-5°C for 1 hr, then further stirred for additional 1 hr at room temperature. A pasty mass obtained which was washed thoroughly with sodium carbonate (10%) to remove unreacted acid, the solid separated by cooling and was dried and purified by recrystallization.

Synthesis of (2-phenyl)-3-carboxamidequinazoline-4-(3H)ones (6)

Compound (5) (2.23, 0.02 mole) and urea (0.6 gm, 0.02 mole), in pyridine 20 ml, the reaction mixture was refluxed for (12 hrs) in oil bath at (180-200°C). Then the reaction mixture was poured in to ice cold water containing conc. HCl. The separated solid was filtered, washed and recrystallized from appropriate solvent table 1.

General Procedure for synthesis of derivatives (7a-c)

2-chloro-N-(substituted phenyl)acetamide (0.01 mole) and (2-phenyl)-3-carboxamidequinazoline-4-(3H)ones [6] (0.01 mole) of in ethanol were refluxed for 24 hrs and kept the product over night, the solid separated on cooling was filtered and purified by recrystallization.

Synthesis of N-(4-acetylphenyl) benzamide (8)

To a mixture of 4-amino acetophenone (0.01 mole, 1.39g) in 25 ml of benzene and benzoyl chloride (0.01 mole, 1.4g) was refluxed for 3 hrs according to literature procedure [12]. After that, mixture was kept overnight. The product thus obtained was recrystallized from appropriate solvent table 1.

Synthesis of N-(4-(3-(4-substituted aryl) acryloyl)phenyl)benzamide (9a9b)

A mixture of substituted benzaldehyde (0.01 mole) and compound (8) (0.01 mole) and (20%) 10 ml NaOH in ethanol (30 mL) was stirred for 24 hrs at room temperature. After that, mixture was poured onto crushed ice to get precipitate. The product thus obtained was purified by recrystallization table 1.

Synthesis of N-(4-(2 mercapto-6-(substituted aryl)-1,6-dihydropyrimidin-4-yl)phenyl) benzamide (10a,b)

A mixture of required chalcone (9a,b) (0.01 mole) and thiourea (0.01 mole) in 1,4 dioxan (10 ml) and acatalytic amount of glacial acetic acid are taken in around bottom flask and heated under reflux for about 24 hrs. The reaction mixture was poured into cold water with stirring, the product was filtered and purified by recrystallization.
Synthesis of N-(4-(2-hydroxy-6-(substituted aryl)-1,6-dihydropyrimidin-4-yl)phenyl)benzamid 

(11a,b)

A mixture of N-(4-(3-4-substituted aryl)acryloyl)phenyl)benzamide (9a,b) (0.01 mole) and urea (0.01 mole) in the presence of KOH (1 gm) in 20 ml ethanol was heated under reflux for 12 hrs, then cooled and poured in ice cold water, the solid mass was obtained by filtration, recrystallization table (1).

Results and Discussion

All compounds were prepared according to the following scheme:

The structures of prepared compounds were identified by FTIR (table 2) and $^1$H NMR spectra (table 3). All results spectral data were in correspondence to expected values. The purity of prepared compounds were checked by using TLC chromatography. The physical properties of compounds are listed in (table 1). Compound (1) was synthesized from benzoyl chloride and urea with dry benzene. The IR spectra of this compound, show the appearance of bands at 3360, 3342, 3225, 3068 and 1674 cm$^{-1}$ which could be due to $\nu$ (NH$_2$, NH), $\nu$ C-H aromatic and $\nu$ C=O amide, respectively. The $^1$H NMR spectra measured in DMSO-d$_6$ at 25 oC revealed a multiplet from 8.41 to 7.64 ppm for aromatic protons, a singlet at 11.58 ppm (N-H$_2$, NH$_2$), and 3.7 ppm (NH$_2$).

Also the reaction between benzoyl chloride and thiourea with dry benzene lead to the formation the compound (2). The spectroscopic observation of compound (2) is given by the appearance of bands at 3360, 3342, 3225, 3068 and 1674 cm$^{-1}$ which could be due to $\nu$ (NH$_2$, NH), $\nu$ C=O aromatic and $\nu$ C=O amide, respectively. The $^1$H NMR spectra measured in DMSO-d$_6$ at 25 oC revealed a multiplet from 8.41 to 7.64 ppm for aromatic protons, a singlet at 11.58 ppm (N-H$_2$, NH$_2$), and 3.7 ppm (NH$_2$). The reaction of benzoyl chloride with anthranlic acid in basic media lead to the formation of compound

Antimicrobial activity

The activity of antibacterial and antifungal were studied by using cup-plate agar diffusion method. The inhibition zones were measured in mm. Amoxilline and Mefenamic acid (500mg/ml) were used as standard drugs for antimicrobial activity. The compounds were screened for antibacterial activity against Klebsiella, Escherichiacoli, Pseudomonas aeruginosa and Staphylococcus aureus in Mullar Hinton agar. The results are shown in the tables 4a, 4b, 4c and 4d. The results obtained by antifungal activity, indicate that compounds (4b, 7a, 11b) show activity against S. aureus and Sepidermidis than E. coli and Klebseilla and compared these results with standard drugs (mefenamic acid and amoxicillin). While only the derivative 10b shows good activity against Klebsiellamoore the activity for standard drugs. The results obtained by antifungal activity, it is found that the compounds (3a, 4a, 7a and 7b) show good activity against condid fungi and these results compared with standard drugs (mefenamic acid and Amoxicillin).
Table (1): FTIR spectra of the synthesized compounds

<table>
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<tr>
<th>No.</th>
<th>v (N-H)</th>
<th>v (C-H) aromatic</th>
<th>v (C=O)</th>
<th>v (C=C)</th>
<th>v (C=N)</th>
<th>Others</th>
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<td>1</td>
<td>3225</td>
<td>3668</td>
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<td>2</td>
<td>3257</td>
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<td>1665</td>
<td>1612-1523</td>
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<td>3283</td>
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<td>3081</td>
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<tr>
<td>7c</td>
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<td>1662</td>
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<tr>
<td>8</td>
<td>3159</td>
<td>-</td>
<td>1602</td>
<td>1597-1539</td>
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</tr>
<tr>
<td>9a</td>
<td>-</td>
<td>-</td>
<td>1597</td>
<td>-</td>
<td>1674(CH=CH)</td>
<td></td>
</tr>
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<td>1514,1336(NO2)</td>
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<td>3265</td>
<td>-</td>
<td>1576</td>
<td>1609</td>
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<td>3259</td>
<td>-</td>
<td>1557</td>
<td>1606</td>
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</table>

Table (2): Proton NMR signals of the synthesized compounds in DMSO-d$_6$

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<th>δ ppm</th>
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</tr>
<tr>
<td>2</td>
<td>12. 33(s, H,NH), 3.89 (s,NH2), 7.7-8.2(m, 5H, phH)</td>
</tr>
<tr>
<td>3a</td>
<td>11.6(s, 2H,NH), 7.84-8.4 (m, 10H, oxa- Hand ph- H)</td>
</tr>
<tr>
<td>3b</td>
<td>12.1(s, 2H,NH), 7.92-8.31 (m, 10H,oxa-Handph-H), 2.1 (s,3H,CH$_3$)</td>
</tr>
<tr>
<td>3c</td>
<td>11.4(s, 2H,NH), 7.7-8.2(m, 9H,oxa-H, ph-H and pyrmi-H )</td>
</tr>
<tr>
<td>4a</td>
<td>11.6(s, 2H,NH), 7.6-8.6(m, 10H ,Thaiz-Handph-H)</td>
</tr>
<tr>
<td>4b</td>
<td>12.1 (s,2H,NH), 7.5-8.4 (m, 10H, Thaiz-Hand ph-H) 2.23 (s,3H,CH3)</td>
</tr>
<tr>
<td>4c</td>
<td>11. 7(s,2H,NH), 7.8 -8.6 (m, 9H, Thaiz H, ph-H and pyrmi-H )</td>
</tr>
<tr>
<td>5</td>
<td>7.55 -8.61 (m, 9H, Aromatic protons)</td>
</tr>
<tr>
<td>6</td>
<td>6. 1 (s,2H,NH2), 7.4-8.56 (m, 9H, Aromatic protons)</td>
</tr>
<tr>
<td>7a</td>
<td>9.33 (s, H,NH), 7.7-8.21 (m, 14H oxa-Handph-H )</td>
</tr>
<tr>
<td>7b</td>
<td>7.6 (s, H,NH), 7.5-8.1 (m, 14H, oxa-Handph-H ), 2.1(s,3H,CH$_3$)</td>
</tr>
<tr>
<td>7c</td>
<td>9.4 (s, H,NH), 7.7-8.50 (m, 13H, oxa-H, ph-H and pyrmi-H )</td>
</tr>
<tr>
<td>8</td>
<td>11.98 (NH),2.3(s,3H ,CH3) 7.7-8.50 (m, 9H, Aromatic protons)</td>
</tr>
<tr>
<td>9a</td>
<td>10.8 (NH),7.8-8.6(m,13H, Aromatic protons) 6.3-6.7(d,2H,CH=CH)</td>
</tr>
<tr>
<td>9b</td>
<td>11.04 (NH), 7.4-8.8(m,13H, Aromatic protons) 6.5-6.8(d,2H,CH=CH)</td>
</tr>
<tr>
<td>10a</td>
<td>11.06 (NH),5.3(s,1H,OH) , 7.6-8.4(m,14H,pyrmi-H and ph-H)</td>
</tr>
<tr>
<td>10b</td>
<td>11.08 (NH), 5.1(s,1H,OH) , 7.7-8.9(m,14H, pyrmi-H and ph-H)</td>
</tr>
<tr>
<td>11a</td>
<td>11.1 (s,H,NH), 12.4(s,1H,SH) ,7.4-8.8(m,14H), pyrmi-H and ph-H)</td>
</tr>
<tr>
<td>11b</td>
<td>11.98 (s,H,NH), 12.6(s,1H,SH) ,7.55-8.90(m,14H, pyrmi-Hand ph-H)</td>
</tr>
</tbody>
</table>
Table 3. Antimicrobial activity of oxazole derivatives represented by % inhabitation against different bacterial and fungal species

<table>
<thead>
<tr>
<th>Compound</th>
<th>Inhibition Zone against (in mm)</th>
<th>Candida albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gram negative</td>
<td>Gram positive</td>
</tr>
<tr>
<td></td>
<td>E.Coli</td>
<td>klebsiella</td>
</tr>
<tr>
<td>3a</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3b</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>3c</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>Mefanamic</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Amoixillin</td>
<td>36</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 4. Antimicrobial activity of thiazol derivatives represented by % inhabitation against different bacterial and fungal species

<table>
<thead>
<tr>
<th>Compound</th>
<th>Inhibition Zone against (in mm)</th>
<th>Candida albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gram negative</td>
<td>Gram positive</td>
</tr>
<tr>
<td></td>
<td>E.Coli</td>
<td>klebsiella</td>
</tr>
<tr>
<td>4a</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4b</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>4c</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mefanamic</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Amoixillin</td>
<td>36</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 5. Antimicrobial activity of quinazoline 4-one derivatives represented by % inhabitation against different bacterial and fungal species

<table>
<thead>
<tr>
<th>Compound</th>
<th>Inhibition Zone against (in mm)</th>
<th>Candida albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gram negative</td>
<td>Gram positive</td>
</tr>
<tr>
<td></td>
<td>E.Coli</td>
<td>klebsiella</td>
</tr>
<tr>
<td>7a</td>
<td>20</td>
<td>11</td>
</tr>
<tr>
<td>7b</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>7c</td>
<td>-</td>
<td>12</td>
</tr>
<tr>
<td>Mefanamic</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Amoixillin</td>
<td>36</td>
<td>9</td>
</tr>
</tbody>
</table>
Conclusion

different heterocyclic derivatives containing thiazole, quinazolin-4-one, oxazol and pyrimidine in structure were prepared and characterized using spectroscopy techniques. four route in the synthesis, the first synthesis of oxazoloderivatives 3a-3c by reaction of benzoyl chloride with urea ,after that cyclization product with (substituted phenyl) acetyl amides.in the same method prepared thiazole derivatives 4a-4c but with thiourea . the third route include preparation of quinazolin 4-one derivatives 7a-7b by reaction of benzoyl chloride with anthranilic acid and reaction product with urea ndcyclization by (substituted phenyl) acetyl amides .in the final scheme synthesis was prepared pyrimidine derivatives 10a-11b.these derivatives have been evaluated in vitro for their antimicrobial activities against several microbes like klebsilliha, Escherichia coli, Psreudomonasaeruginosa and Staphylococcus aureus, addation of antifungl activity.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Chemistry, College of Science-University of AL-Mustansiriyah- Baghdad-Iraq and all experiments were carried out in accordance with approved guidelines.

References

15. Tolba M S , KamalEL-Deen A M , Ahmad M , Hassanian R , Farouk M. Synthesis and antimicrobial
activity of some new thienopyrimidine derivatives.


Risk Factors related to Sudden infant death syndrome (SIDS)

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Abstract

Sudden infant death syndrome (SIDS) is a phenomenon of unknown cause among infants between 1 month and 1 year of age”. Methodology: descriptive design conducted through September 2018 to the end of March 2019, mothers attended health centers in Al- Hilla City, who consists of (120 meters). Data were collected through using SIDS_QA-508 after doing some adjustment on the questionnaire, The questions that used in collecting data were checked out for any mistake or error and scrutinized, Data of the 120 participant’s were entered and analyzed by means of the statistical package for social sciences (SPSS) .The results indicated that the children age (65.0%) was less one year aged, (41.7%) mother’s educational attainment with primary school. (94.2%) with pre-term pregnancy, (74.2%) were married, (77.5%) from the population present their satisfaction concerning monthly income and (55.0%) had health problems through their pregnancy. Most mothers have low knowledge regarding abrupt baby death, which correlated for mother’s age, sources of knowledge as weak correlation. Recommendations: Hospital staff should instruct parents who, in turn, must emphasize to secondary caregivers that the baby must always be placed to sleep on the back and never on the tummy, since prone sleep increases the risk of SIDS.

Key words: Infant, death syndrome

Introduction

“Sudden infant death syndrome (SIDS)” is an unknown reason of death among infants within the first year, which has a critical link with abdominal sleeping since 1965. The pathophysiology of SIDS is incompletely understood, but the critical period of newborn development exposed him to triggers and unexpected death could be allied to immaturity of respiratory control which cause hypoxemia and long apnea(Coverstone, 2019, Wilson, 2017). “The philosophies of safe sleeping practices among infancy are not well recognized, however, a considerable body of evidence supports back sleep. As long as, many sudden baby death risk factors are linked to certain unexpected influences occurring during sleep, “the Amerian Academy of Pediatrics” expanded its strategy to comprehend diversity of harmless sleep performs that can diminish hazard for sleep-related infant death to endorse discouraged bed sharing and smoking, and authorized the use of the supine position and fitting surfaces for all sleep, breastfeeding, and adequate prenatal care. Risk factors of “SIDS” are complicated by social and economic factors, whereas, in United States, the young, poor, and minority women are at elevated risk for this problem 3, in addition to lack of prenatal care, low socioeconomic status, smoking, and low birth weight babies are also associated with increased risk. It was influenced by community norms and cultural practices 4, in particular infant sleep practices, moreover, multiple epidemiologic studies have shown a strong relationship between paternal smoking and SIDS 6. These unsafe issues increases the infant mortality rate in the world is 29.5 (2017) Iraq IMR is 23.3 per 1000, Iran is 12.1 per 1000, Jordan is 13.8 per 1000, Egypt is 17.5 per 1000 (2013)” — a proportion greater than most other developed countries abrupt death and suffocation account for more than half of all unexpected infant, which considered as the
leading causes of post neonatal infant death in the US. Aitken’s grandmother and other caregivers can effect on mother’s decision in selection of the sleep position and their beliefs about infant comfort and other traditions may also affect attitudes to risk reduction. Researcher’s always focuses on reassuring the non-risky behaviors, specifically focusing on supine position and sleep location. Over and above, interested in differences between grandmothers’ behavior in their own homes compared to the home of the parent, along with, whether safe sleep practices were associated with beliefs in sleep safety. So, the present study aims to assess the risk factors related to “Sudden Infant Death Syndrom” through the first year of life.

Methodology

II.1: The design and setting of the study; A descriptive study was conducted on a number of mothers who lost their babies with less than one year age in Babel province during the period from October 2018 to April 2019.

The study was conducted with mothers who visit “MCH” centers, which include Shemali, Al Huda, Meditative Model, Qasim, Hashemite.

II.2: Study sample: Mothers included were (120 mothers) who attended health centers in Al- Hilla City

II.3: Instrument of the Study: Data were collected through using “SIDS_QA-508 (NIH Pub. No. 07-7202 June 2007)”. The researchers used a standardized questionnaire to identify the risk factors for sudden and pre-programmed death syndrome for the same purpose in European and Arab countries.

The questionnaire consisted of two parts: the first related to demographic information and the second contains (40) bubbles allied to the subject of the study. The internal correlation coefficient between these paragraphs was calculated and the results were shown (0.79). Few modifications done on it, like reducing the scale from five to three scales, and delete some points from demographical (“Increasing the number of scale categories did not necessarily improve performance”).

The sample are (120) mothers, selected from more than 3000 visitors to “MCH” centers, the information was collected by interviewing mothers who lost their children under the age of the year.

The researchers interviewed mothers and explained the dimensions and importance of their participation in the research and the results of this study, in addition to helping them to understand some of the issues related to the questionnaire.

II.4: Data Collection: After the collection of information according to the questionnaire, which was approved by the “Health Directorate of Babylon”

II.5: The Statistical Analysis: The researchers collecting data construct a database on the (SPSS, version 24,2016) statistical system and analyzed; using descriptive statistics to calculate the frequencies, percentages and standard deviation.

II.6: Ethical consideration: All participants were informed fully about current study and its aims and voluntary verbal consent was obtained from the participants in order to participate in the study. Besides, it has been taken into account the confidentiality of information obtained.

Results

Table (1) shows the risk factors related to “SIDS”

<table>
<thead>
<tr>
<th>Was the baby crying, strong after birth?</th>
<th>Freq.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>78</td>
<td>65.0</td>
</tr>
<tr>
<td>Yes</td>
<td>42</td>
<td>35.0</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>100.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>The chid was given oxygen after birth</th>
<th>Freq.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>53</td>
<td>44.2</td>
</tr>
<tr>
<td>Yes</td>
<td>67</td>
<td>55.8</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>100.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Has the baby sharing your bed through feeding</th>
<th>Freq.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>50</td>
<td>41.7</td>
</tr>
<tr>
<td>Yes</td>
<td>70</td>
<td>58.3</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>100.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of feeding</th>
<th>Freq.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breastfeeding</td>
<td>60</td>
<td>50.0</td>
</tr>
<tr>
<td>Bottle feeding</td>
<td>42</td>
<td>35.0</td>
</tr>
<tr>
<td>Mix</td>
<td>18</td>
<td>15.0</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>100.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Feeding the baby during sleep</th>
<th>Freq.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>59</td>
<td>49.2</td>
</tr>
<tr>
<td>Yes</td>
<td>61</td>
<td>50.8</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>100.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Baby choked during feeding?</th>
<th>Freq.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>49</td>
<td>40.8</td>
</tr>
<tr>
<td>Yes</td>
<td>71</td>
<td>59.2</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>100.0</td>
</tr>
</tbody>
</table>

This table indicated that the factors effect “RDS” are ,(65.0%) of the baby had not cry strongly after the birth, (55.8%) of the children were given oxygen after birth, while (58.3%) of the babys sharing mothers’ bed throughout feeding or sleeing, (50.0%) were fed from breast, (50.8%) feeding the baby during sleep and (59.2%) the baby were choked during feeding. The risk factors of “SIDS” are complicated by societal
and financial factors; which almost allied with lack of prenatal care, low socioeconomic status, smoking, and low birth weight. In addition to cultural practices have a great impact on infant sleep practices. “Regardless the age of a new mother, intergenerational transmission of childrearing practices is common.” Table (1) revealed that nearly half of the sample included in the study are educated primarily, (41.7%) of the mothers with poorly follow-up through pregnancy with non suitable safe practices, which effect on their babies weights to have a low birth baby which constituting (52.5%), whereas (94.2%) as preterm delivery, and (45%) of them exposed to smoking besides to other indicators could be clearly detected in same table. (35%) of the babies crying strong along the night, (50%) of them feed just from breast with more than half, despite the fact (58%) were sharing mother’s bed for the period of sleeping; which considered as a chief cause for “SIDS”. In 2015 US, more than half (61.4%) of the respondents reported in bed sharing with their infant, and(37.0%) reporting “rarely or sometimes” and 24.4% responding “often or always” bed sharing, Self-report of any bed sharing varied by state, ranging from 49.0% in West Virginia to 78.9% in Alaska. The prevalence of bed sharing varied by maternal characteristics, gestational age at birth, and breastfeeding at 8 weeks postpartum being elevated. Bed sharing prevalence was higher among respondents in American Indians/Alaska Natives, non-Hispanic blacks, or Asians/Pacific Islanders compared with non-Hispanic whites or Hispanics. The population effected by many beliefs from their mothers when mentioned that they must to share because the babies in majority (59.2%) choking through night feeding. CAPTMinn S, (2013) stated that the reasons of practicing or choosing non supine sleep positions with their sharing reported by parents in this study; were for infant preference, comfort, and fear of choking or safety on supine position. Conversely, those that chose supine sleep position did so for safety reasons, based on advice and recommendation from their health care providers and their knowledge that this position reduced the risk for “SIDS”. Similarly, The participation of nurses in the care of newborns reduces the risk of deformities in the head while lying down.

**Conclusion**

The study concludes that infants less one year aged, their weight less than two Kg, estimating equal sex between male and female, as well as, mother’s educational attainment with primary school most mothers had pre-term babies in majority, they had not health problems during pregnancy and complications occurred during childbirth, delivered by normal birth, the baby had not cried, strong after the birth and feeding the baby during sleep which get choked during feeding.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of Nursing, University of Babylon, Iraq and all experiments were carried out in accordance with approved guidelines.

**References**


Design and Implementing System to Study Environmental Noise Pollution Using Microcontroller

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1University of Mustansiriyah/ Engineering Faculty/ Computer Engineering Department/ Baghdad/ Iraq,
2University of Baghdad/ Science Faculty/ Computer Science Department/ Baghdad/ Iraq

Abstract

This paper proposed a system and presents its result obtained in a study on environmental noise pollution in the city of Baghdad, Iraq. The sensing system is built on Atmega128 and CC2420 platform. We designed and implemented a sensor function for wireless sensor network application using special network named (WSN) for measuring environmental acoustic noise.

Based on the readings obtained became clear that 90 % out of the locations during the peak time have equivalent sound level over 75 dBA, and 60 % out of the total number of locations measured display during the normal time equivalent sound level over 75 dBA.

Key words - Pollution, environmental monitoring, Noise, WSN, T-Test

Introduction

The effect of noise pollution on human health and his welfare is recently considered as an important and vital issue which encourages scientists and interested agencies all over the world, such as World Health Organization (WHO), to conduct more researches concerning assessment of its levels and harmful effects. Sometimes defined in common speech the environmental noise as unwanted sound. The European Environment Agency defines environmental noise as ‘unwanted or harmful outdoor sound created by human activities, including noise emitted by means of transport, road traffic, rail traffic, air traffic, and from sites of industry activity’

Noise in industries such as construction equipment, electrical machines, loudspeakers, hawkers, vehicle movement, railroad work, and airplanes in the airports which cause an increased blood pressure and an irritation decreasing the work efficiency. It is therefore of utmost importance that excessive noise is controlled. We use the term decibels (dB) to explain sound intensity. World Health Organization (WHO) has illustrated that the most appropriate noise level not bigger than 75 dBA to prevent hearing loss. Any level above 75 dBA will effect on human ear with time. World Health Organization (WHO) recommendations that required noise level over 75 dBA in streets and public places (outdoors) leads to weakness of hearing. It is undesirable to exceed noise level the permissible limit, because it is harmful to human health and causes an inability to concentrate and nervous tension, therefore government institutions must develop appropriate solutions to reduce the pollution by noise and its effects on people’s lives. The European Directive 2002/49/EC requires all Member States to provide accurate noise level mappings of inhabited areas with more than 250,000 people and make this information publicly available via web-interfaces of appropriate quality. This article aims to design a digital sound intensity measurement system capable of sensing sound-frequency controlled by microcontroller & programming in C++ language. The main application areas of sound intensity sensing include different streets in Baghdad at two different times. We chose 16 of the most important areas in Baghdad (Al-Karrada, Al-Mansour, Al-Sinak, Al-Sadr City, Bab Al-Moatham, Bab Al-Sharqi, Al-Mustansiriyah, Al-Dora, Hae Al-Khadhraa, Al-Sha‘ab) to measure sound intensity and to compare measured results with permissible parameters. It was measured in two separate periods, one at the peak time when end of the working hours (1:00 pm – 4:00 pm) and the other in the morning (9:00 am - 12:00 pm). This work focuses primarily on the establishment of wireless environmental monitoring systems proposed to measure noise pollution by sound within cities. There are many requirements that need attention when designing wireless sensor networks.
when talking about environment in cities. In 8, System designed for monitoring environment pollution should be flexible, reliable and independent. applied a test network based on the TinyNode 8 MHz platform with a XE1025 transceiver 868MHz to observe a hydrological model of the Swiss Alps. in order to monitor environment, authors In 12 proposed a WSN framework: a Distributed Signal Processing infrastructure (SbDSP) based on the sensor to deal with sound data in a distributed manner. Anyway, a detailed application did not included in the sheet. The work done in 15 may be the closest to our project. In their work, the Tmote Sky platform 16 was applied with deploying sensors to measure traffic noise on the roads. Through the measured data, the number of vehicles and the number of different species can be calculated. The authors stress that the process of measuring noise in wide-scale using the WSN solution is possible. However, the accuracy of their measurements was not illustrated, leaving the contract calibration open, and the sensor sampling rate was determined at 8 kHz due to the reduction of the CPU / ADC. Examine the effects of noise pollution in other research has been undertaken in residents of Birj and its airport. The purpose of this research is to apply the descriptive study of the group, where took data at a specific time in a cross-sectional study. The purpose is to use 144 questionnaires by Morgan table distributed to 500 peoples living in those analyzed areas. 2 These studies have been done in narrow spaces that facilitate the handling and control the physical components and human, which confirms on the importance of introducing technology and programming to collect information and obtain results.

Method of Sampling

Our objectives were about measuring noise pollution levels in different areas with different traffic flow at different periods of time. Sound levels were measured using a controller consisting of microcontroller, sound sensor, and transceiver. In each location, the device is turned on for 10 minutes as a playback time and for a continuous measurement period of 2 to 3 hours during which several sound readings are recorded, sent and automatically saved to the computer, then calculate sound intensity. Noise levels of environmental pollution were measured in two different periods of the day to study the impact of human activity and vehicular traffic on the street. The first period was during the morning (9:00am-12:00pm). This period is characterized by relatively low traffic activity for cars and pedestrians because they are present in their jobs. The second period was during which noise measurements were carried out at the test hour (1.00pm-4.00 pm). This period is characterized by the maximum traffic of vehicles and pedestrians due to continuous working hours and the momentum of cars and other industrial and commercial activities in those streets. We correlated the obtained information of WSN sensor with XD-58C sensor to declare the effect of noise pollution on heart rate. We did the test on different levels of people were documented properly and collected in an excel File.

- Body Area Network (BAN) Implementation

For implementing such network we used XD-58C Pulse Sensor for arterial pressure and heart rate was used. This module is designed to plug-and-play heart-rate sensor for Arduino, any medical equipment that are measure heart rate may use this module. It can be worn on finger. its need 5 volts & 4 mA to work. It has open-source monitoring app that graphs pulse in real time. The high levels of pollution by noise cause every year a lot of heart attacks; therefore should be monitored heart health frequently.

![Pulse Sensor Amped Visualizer v11](image)

To sketch the heartbeat waveform and check heart rate, we used software program “Pulse Sensor Amped”.

Collecting Data Method

A plan has been created to check research objectives and testing devices. The test was applied on group of 10 human; 5 males and 5 females them ages is between 25 and 40 years. They were subjected to noise level equal to 80 dB in average and for 20 minutes. The XD-58C sensor was hanged on a hand of each person during the test to measure blood oxygen saturation, and heart rate. There was a variation in the heart rate and changes in blood oxygen saturation levels when most of the people who were tested after exposing to noise. from this group,
one person had a significantly increased blood oxygen saturation and heart rate levels, felt in stress, and he had some pain in one ear. The results showed high heart rate and blood oxygen saturation levels values on the selected group which confirm the findings data published by the World Health Organization (WHO) regarding the harmful effects of noise pollution on human health. Figure (2, 3) shows changing in heart rate levels before and after exposure to noise.

We read sound levels, which wrote linearly through analog port of Arduino and then send it by WSN; at the base node receive data and recorded it to a computer automatically. WSN is a modern technology that is currently applied in a variety of fields including, the environment, natural disasters, public safety, Government, Health and intelligent buildings and machine surveillance. The sensor network contains a specific set of sensor nodes and a gateway connected to an external communications network. WSN networks are useful as well it considered low cost and low power electronic components. We had used LM386 in order to measure the sound level with a KY-038 microphone sound sensor; this design had used in order to reduce the
power consumption where the circuit works with low voltage 5V. After received a signal (the sound), converts to a
digital value in order to be dealt with by the computer.

\[
R = \frac{V_{\text{I, max}}}{N_{\text{comb}}} 
\]

Where: \(V_{\text{I, max}}\) is the maximum input voltage defined as 5V; \(R\) is the resolution; the obtained
resolution value is \(R=4.9\text{mV}\); \(N_{\text{comb}}\) is the number of combinations for 10 bits, it means \(2^{10}=1024\).

Acquisition is used for converting to analog:

\[
V_A = \frac{V_{\text{I, max}} \cdot V_S}{N_{\text{comb}}} 
\]

Where: \(V_A\) is for converting voltage from analog to digital; \(V_S\) is a pin in arduino obtained
the sound level meter voltage in analog. Because the sound level meter has a DC output where
1dB of noise represents 10mV it is obtained:

\[
V_S = \frac{S}{V_{aq}} 
\]

Table 1 - Noise Risk Zone Criteria Adopted To Be Used In This Study. 1

<table>
<thead>
<tr>
<th>Intensity of noise in dB(A)</th>
<th>Zones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 66</td>
<td>Safe</td>
</tr>
<tr>
<td>66-71</td>
<td>Tolerable</td>
</tr>
<tr>
<td>71-76</td>
<td>moderate risk</td>
</tr>
<tr>
<td>76-81</td>
<td>high risk</td>
</tr>
<tr>
<td>81-86</td>
<td>High risk</td>
</tr>
<tr>
<td>Greater than 86</td>
<td>extremely high risk</td>
</tr>
</tbody>
</table>

We have selected some residential and commercial districts to measure sound intensity in two periods of the day.
The result that we get it as below:

In general, sound intensity had obtained indicate an increase in sound intensity, which negatively effects on the
lives of people in this city, therefore its need to develop procedures to reduce this effect. By comparing the result we
obtained with the default values in table (1) we got the following table:

Table 2 - Classification of district of Baghdad city during active hours (9am–4pm)

<table>
<thead>
<tr>
<th>Street</th>
<th>Noise Value</th>
<th>Zones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al-Karada</td>
<td>82.03</td>
<td>high risk</td>
</tr>
<tr>
<td>Bab Al-sharqi</td>
<td>86.68</td>
<td>extremely high risk</td>
</tr>
<tr>
<td>Bab Al-Muazam</td>
<td>83.43</td>
<td>high risk</td>
</tr>
<tr>
<td>AL-sinak</td>
<td>83.95</td>
<td>high risk</td>
</tr>
<tr>
<td>Mansor</td>
<td>79.73</td>
<td>moderate risk</td>
</tr>
</tbody>
</table>
Table 2 - Classification of district of Baghdad city during active hours (9am–4pm)

<table>
<thead>
<tr>
<th>District</th>
<th>Noise Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Camp Sara</td>
<td>82.03</td>
</tr>
<tr>
<td>AL-shurja</td>
<td>83.95</td>
</tr>
<tr>
<td>AL-Bayaa</td>
<td>79.73</td>
</tr>
<tr>
<td>Hai Al-Xadhraa</td>
<td>74.05</td>
</tr>
<tr>
<td>Al-Shaab</td>
<td>80.53</td>
</tr>
<tr>
<td>Sader city</td>
<td>82.80</td>
</tr>
<tr>
<td>Al-Dora</td>
<td>76.70</td>
</tr>
<tr>
<td>Al-Adhamia</td>
<td>76.70</td>
</tr>
<tr>
<td>Zayona</td>
<td>74.80</td>
</tr>
<tr>
<td>Zaaafarania</td>
<td>81.65</td>
</tr>
<tr>
<td>AL-mustansiriyah</td>
<td>76.28</td>
</tr>
</tbody>
</table>

Table 3 - T Test analyzing noise intensity in commercial streets

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal time</th>
<th>Peak time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>75.4188</td>
<td>89.9563</td>
</tr>
<tr>
<td>SD</td>
<td>2.8578</td>
<td>2.1882</td>
</tr>
<tr>
<td>SEM</td>
<td>1.0104</td>
<td>0.7736</td>
</tr>
<tr>
<td>N</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

P value

<table>
<thead>
<tr>
<th>P value</th>
<th>Mean</th>
<th>95% confidence interval of this difference</th>
<th>T – value</th>
<th>( D_f )</th>
<th>standard error of difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>less than 0.0001</td>
<td>-14.54</td>
<td>-16.24 -12.8322</td>
<td>7.12</td>
<td>20.1586</td>
<td>7</td>
</tr>
</tbody>
</table>

The T-test [Table 3 & 4] showed that there were strong statistical differences (\( P < 0.0001 \)) between the noise levels during the two measurement periods as shown in (Figures 3). These data confirm that the traffic activity during the period of the beginning and end of official working hours considered the main source of noise pollution in the city of Baghdad. The statistical comparison of the results with respect to the activity of some streets indicated that there is no large difference between the results of the two measured periods, as they are areas of cars and passers-by and are considered permanent crowded areas in all active hours of a day.

Conclusion

Based on the readings obtained with the use of Arduino microcontroller and voice sensor, we found the percentage of sound pollution in the different areas of Baghdad. Became clear that Based on the readings obtained that 90% out of the locations during the peak time have equivalent sound level over 75 dBA, and 60% out of the total number of locations measured display during the normal time equivalent sound level over 75 dBA, and 90% of them over 75% during peak time. The most Baghdad area in the sound pollution is Bab Al-Sharqi (86 dBA), While lowest Baghdad areas in
sound pollution level were districts with low density population such as Hay Al-Khadraa, where it reached (74 dBA). The used design have accurate readability, low cost with ease of use, which makes it easy for us to compile readings, analyze them and produce satisfactory results.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the University of Mustansiriya, Iraq and all experiments were carried out in accordance with approved guidelines.

**References**


Nurses Staff Knowledge Regarding Standard Hand Hygiene Precautions in Rania City/Kurdistan Regional Government of Iraq

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College of Nursing, University of Raparin

Abstract

Objectives: -To assess nurses staff level of knowledge regarding standard hand hygiene precaution, and to find out the association between their level of knowledge and some of socio-demographic characteristics such as (gender, and level of education).

Method: Quantitative design, a descriptive study was carried out at Rania City. To achieve the objectives of the present study, non-probability purposive sample was used. The study sample was (66) nurses who were providing nursing services at two hospitals (Rania teaching hospital and Rania maternity and pediatric hospital). 10 of samples were excluded for a pilot study.

A questionnaire was constructed by the researcher for the purpose of the study technique and it was used as a tool for data collection. The data were collected through using an interview technique (face to face) approach.. Data were analyzed through the application of descriptive statistical analysis, such as: (frequency, percentage, mean of scores), and inferential statistical analysis (Pearson’s chi-square and correlation coefficient), by using the statistical package of social sciences (SPSS) version (20).

Results: The study indicated that the most of samples aged between 32-36 years old and represents 28.6% of the study samples. In addition, the highest percentages of the nursing staffs were female 73%. Concerning of the educational level, more than half of nurses graduated from the medical institute 66 %. And the largest study sample have 6-10 years of experiences 28.5%The findings of the study reveal that the nurses have a good level of knowledge about hand hygiene (HH) and appropriate time to do HH. The majority of the stay sample routinely used HH and they disinfectant their hands either by washing hands with water and soap or use alcohol-based jell for, while some of the nurses don’t wash their hands either because they are busy, they forget to do it or even the products are not available or not easy to reach. The study also approves that there was a statistically no significant association between the knowledge of nurses and gender and years of experiences.

Keyword: Hand hygiene, Hand washing, Alcohol-based hand rub, Knowledge, Nurses

Introduction

Nurses do hand hygiene even by washing hands with water and soap or using alcohol-based hand rub to prevent spread of infection to them or even to patients. With that there are many moments that the nurses do not do it either because they are busy, they forget to do it or the products are not available or not easy to reach. In addition, they do not wash their hands correctly because of lack of knowledge about standard HH. Hand hygiene is a simple, low cost and an effective way to prevent separation of infections. Cleaning your hands can prevent the spread of germs and save the nurse and the patient lives. Hand hygiene refers to either hand washing with antimicrobial soap or hand disinfecting with an alcohol-based hand-rub. The aim of hand hygiene is to remove dirt and limit the microbial counts on the skin, to prevent cross transmission of pathogens between patients 1. The New England Journal of Medicine reports that a lack of hand-washing remains at unacceptable levels in most medical environments, with large numbers of doctors and nurses routinely forgetting to wash their hands before touching...
patients, thus transmitting microorganisms. One study done in ICUs in Michigan (2003), showed that proper hand-washing and other simple procedures can decrease the rate of catheter-related bloodstream infections by 66 percent. The World Health Organization (WHO) has issued guidelines for procedural hand washing in order to reduce the prevalence of hospital associated infections, but lack of knowledge amongst health care workers is associated with poor compliance. To reduce the spread of germs, it is better to wash the hands or use a hand antiseptic before and after tending to a sick person. All staff in hospital should deal with hand hygiene actively because this is one of the easiest ways to help keep patients as safe and healthy as possible. So there is a need to check up nurses’ knowledge and awareness toward hand hygiene periodically in order to ensure prevent spared of infection in health care setting and to stop patients and health care workers from getting infections.

Method

A quantitative design, descriptive study was carried out in the Rania city from August 2, 2018 to the end of March 2019. In order to assess nursing staff level of knowledge regarding standard precaution (Hand hygiene) in Rania city. Rania is a city located in the Slimani Governorate Kurdistan Region/Iraq. A non-probability purposive sample of (66) nurses at the Rania teaching hospital and maternity and pediatric hospital. Who were providing nursing services at hospitals, 10 of samples excluded for a pilot study. Through an extensive review of relevant literature, and previous studies a questionnaire was constructed by the researcher for the purpose of the study and it is used for data collection. The questionnaire consisted of three parts: 1st part includes socio-demographic characteristics of nurses, staff, 2nd part consists of (7) question about HCWs compliance regarding hand hygiene, and 3rd part includes (18) items, which include (2) sections. Section (1) consists of (11) items, about knowledge of study samples regarding the hand hygiene, section (2) consists of (7) items, about the most appropriate timing of hand hygiene actions that prevent transmission of germs to the patient. The data were collected through the use of interview technique (face to face). Items were measured by using two levels of (Likert) scale and rating as, the Yes answer (2), No answer (1). To content validity of the tool was established through penal of (5) experts. Reliability of the questionnaire was determined through the use of (test-retest) technique by using Pearson’s coefficient of correlation formula. This means that the questionnaire format was adequately reliable as a tool for data collection. Data were analyzed through the application of descriptive statistical analysis, such as: frequency, percentage, mean of scores, Pearson’s chi-square and correlation coefficient and independent sample test), in addition use the statistical package of social sciences (SPSS) version (20). The mean of the score (1-1.33) was considered a low level of knowledge, (1.34 - 1.67) considered a moderate level of knowledge, and (1.68 – 2) considered a good level of knowledge.

Results

Figure (1). When nurses worked with his/her colleague and he/she forgot to disinfect their hands before touching the patient.
The bar indicate when the nurses worked with his/her colleague and he/she forgot to disinfect their hands before touching the patient, in what percent he would remind him (28.57%) percentage of nurses about (75-100%) remind his colleagues, (21.43%) says they will remind them in a percentage between (25-50%). (33.93%) give a percentage between (50-75%). In addition to (8.93%) says I never be reminded him to do hand hygiene and (7.14%) give between (0-25%).

Table (1): Level of knowledge of the study sample regarding the hand hygiene action with (2) levels of scale and frequency, percentage, mean of scores, and comparative significant.

<table>
<thead>
<tr>
<th>No</th>
<th>Items</th>
<th>Yes</th>
<th>No</th>
<th>MS</th>
<th>Comparative significant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>F</td>
<td>%</td>
<td>F</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>Hand hygiene (HH) reduces chances of spreading infections</td>
<td>56</td>
<td>100%</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>2</td>
<td>Perform HH by means of hand rubbing or hand washing</td>
<td>49</td>
<td>87.5%</td>
<td>7</td>
<td>12.5%</td>
</tr>
<tr>
<td>3</td>
<td>Handwashing: washing hands with plain or antimicrobial soap and water</td>
<td>53</td>
<td>94.5%</td>
<td>3</td>
<td>5.5%</td>
</tr>
<tr>
<td>4</td>
<td>Alcohol-based handrub formulation: an alcohol-containing preparation</td>
<td>48</td>
<td>85.25%</td>
<td>8</td>
<td>14.25%</td>
</tr>
<tr>
<td></td>
<td>for application to t for reducing the number of viable microorganisms on the hands</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Wearing gloves did not replace the need for HH</td>
<td>36</td>
<td>64.25%</td>
<td>20</td>
<td>35.75%</td>
</tr>
<tr>
<td>6</td>
<td>Washing hands when visibly soiled, otherwise use hand rub</td>
<td>28</td>
<td>50%</td>
<td>28</td>
<td>50%</td>
</tr>
<tr>
<td>7</td>
<td>Duration of the washing hands procedure when visibly</td>
<td>44</td>
<td>78.5%</td>
<td>12</td>
<td>21.5%</td>
</tr>
<tr>
<td></td>
<td>soiled 40-60 seconds</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Duration of the hand rub procedure: 20-30 seconds</td>
<td>42</td>
<td>75%</td>
<td>14</td>
<td>25%</td>
</tr>
<tr>
<td>9</td>
<td>HH contributes significantly to keeping patients safe</td>
<td>55</td>
<td>98.25%</td>
<td>1</td>
<td>1.75%</td>
</tr>
<tr>
<td>10</td>
<td>Hand need to be dried after hand hygiene</td>
<td>42</td>
<td>75%</td>
<td>14</td>
<td>25%</td>
</tr>
<tr>
<td>11</td>
<td>Jewelry, ring, and watch should be removed during washing</td>
<td>47</td>
<td>84%</td>
<td>9</td>
<td>16%</td>
</tr>
<tr>
<td>12</td>
<td>HH must be done immediately before aseptic procedure</td>
<td>44</td>
<td>78.5%</td>
<td>12</td>
<td>21.5%</td>
</tr>
<tr>
<td>13</td>
<td>HH must be done after handling objects and device such as soiled</td>
<td>51</td>
<td>91%</td>
<td>5</td>
<td>9%</td>
</tr>
<tr>
<td></td>
<td>linen, trash and equipment.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>HH must be done after contact with blood, body fluids</td>
<td>56</td>
<td>100%</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>secretions or excretions, mucous membrane.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

G.I= Good Knowledge M.I= Moderate Knowledge L.I= Low Knowledge M.S= Mean of score S= Severity
This table indicated that the mean scores were good for knowledge of study samples regarding the hand hygiene actions and most appropriate timing that prevent transmission of germs to the patient.

**Table 2. The association between nurses level of knowledge regarding hand hygiene and gender**

<table>
<thead>
<tr>
<th>Independent Samples Test</th>
<th>Levene’s Test for Equality of Variances</th>
<th>t-test for Equality of Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>Sig.</td>
<td>t</td>
</tr>
<tr>
<td>Knowledge of nurses</td>
<td>Equal variances assumed</td>
<td>0.049</td>
</tr>
<tr>
<td></td>
<td>Equal variances not assumed</td>
<td>-0.897</td>
</tr>
</tbody>
</table>

This table shows there is no significant association between knowledge of nurses regarding hand hygiene and gender at level of P value ≤ 0.05.

**Table 3. The association between nurses level of knowledge regarding hand hygiene and years of experiences Pearson correlation**

<table>
<thead>
<tr>
<th></th>
<th>Years of Employment</th>
<th>Knowledge of nurses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Years of employment</td>
<td>Pearson Correlation</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>56</td>
</tr>
<tr>
<td>Knowledge of nurses</td>
<td>Pearson Correlation</td>
<td>0.066</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>56</td>
</tr>
</tbody>
</table>

This table shows there is no significant association between knowledge of nurses regarding hand hygiene and years of employment at level of P value ≤ 0.05.

**Discussion**

Hand hygiene is one of the essential ways to prevent or reduce the spread of infection in the health care setting. Periodically assessing knowledge of nursing staff regarding hand hygiene is an important ways to provide them with update information. In relation to the sociodemographic characteristics of the nurses’ the results show more than a quarter percentages of the nurses their ages between (32-36) years old. More than two-third of the study sample were female, due to Rania city has two hospitals; (Rania teaching hospital and Rania maternity and pediatric hospital). Most of the nurses in maternity and pediatric hospital were female. Concerning the level of education, more than half percentage of the samples are graduated from the medical institute, at the
same time only a small percentage of the nurses were graduated from nursing college 12%. Regarding the years of experiences, above the quarter percentage of nurses’ has (6 - 10) years, while only five percentages of nursing staff have (16 – 20) years’ experience. The result is an agreement with the Alireza Sharif study, the result of the study shows most of the nurses (38.5%) had working experience 5-10 years 6. But another study done in Mozambique which titled (Cold chain management: Knowledge and Practice in PHC facilities) that one-third of workers had between (2 to 5) years which constituted 32 % 7. Concerning source of information, more than 70 % of nurses had a self-reading either from reading books or from internet to increase or update their knowledge about HH. In addition, more than half of nurses took the training course about HH. The hospital administration, always tried to help their nursing staff to participate in course training in order to increase the staff’s knowledge and experiences about hand hygiene. While the diagram 1 shows that the more than seventy percentage of the study sample routinely used hand hygiene either by washing hands with water and soap or use alcohol-based handrub, but nearly 30 % did not do hand hygiene routinely. This is not acceptable in hospitals because maybe they become source of transmission of infection. Healthcare workers’ hands are the source for the carrying of pathogens from patient to another and within the healthcare environment. The health care worker commitment to the highest degree of practices remains low 10. The study also shows the reason that makes the nurses did not do hand hygiene due to they are too busy, out of product or the product are way or located in an inconvenient location to get in time. In addition, they forget to do HH sometimes. Even though these excuses are not reasonable for nurses or other health care worker, they need to do hand hygiene in order to prevent or reduce the transmission of infection in healthcare facilities. Health experts say poor hand hygiene is one of element in hospital-borne infections that kill tens of thousands of Americans each year. One every 25 patients in U.S. hospitals get a hospital-acquired infection as part of his or her care despite modest progress in controlling those pathogens inside medical facilities, the Centers for Disease Control and Prevention (CDC) reported in its most comprehensive look at a stubborn and dangerous health care problem 11. The result of this study is consist with the study done in teams in eight hospitals used secret observers, to identify the causes of hand hygiene noncompliance. Data from the eight hospitals revealed 41 different causes of noncompliance, which were some of them were healthcare worker forgot, inconvenient placement of hand rub dispenser or sink, broken dispenser or sink and healthcare worker was too busy 12. The result of this study also shows depicted that no significant association between knowledge of nurses regarding hand hygiene with gender and years of experiences. The mean knowledge score was not associated with gender (p=0.82), and years of employment (p=0.63). This may back to some reasons like the small percentage of study samples, and this is out of hand of the researcher due to Rania city has only two hospitals, and only nurses includes in the study. Another reason is the most of the study samples have the same level of education (most of them graduated from the institute). There is consistent with a study done about the knowledge of hand hygiene among the healthcare workers of two teaching hospitals in Mashhad. The result shows there was no significant difference in the knowledge level of the participants who had received formal training in hand hygiene and those who had not. Also, the mean knowledge score was not associated with age, gender, department, and the profession 13.

**Conclusion**

According to the results of the study, nurse have good knowledge regarding HH, but they are not routinely aware to use HH due to they are too busy or because out of disinfect product or they forgot to do that. In addition, most of them, they did not remind other to disinfect their hands before touching the patient in case they forget to do that. Finally, the findings of the study also show there is no significant association between nurses’ knowledge regarding HH with gender, and years of experiences.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of Nursing, University of Raparin, and all experiments were carried out in accordance with approved guidelines.

**References**

2. Goldmann D. System failure versus personal
accountability—the case for clean hands. New England Journal of Medicine, 2006;355(2): 121-123.


Risk Factors Increasing Prevalence of Type 2 Diabetes Under The Age of 40 Years Attending Al-Diwanyia Teaching Hospital

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1Department of Community and Family medicine, University of Al-Qadisiyah, College of Medicine, Iraq

Abstract

The present case control study of Iraqi people living in Al-Diwanyia city to identify the attributable risk factors that leads to type 2 diabetes in younger age groups and the correlation of the most prevalence risk for developing diabetes. The study was designed to be a case control study includes 315 individuals, 150 is the number of controls, and the rest of the participants are patients that have been diagnosed by diabetes mellitus type 2 before or at age of 40 years. All these participants attended outpatient clinics at Al- Aldiwanyia teaching hospital in Al-Dewaniyah province/ Iraq. The beginning of data collection was dated on the January 2019 and ended on June 2019. Mean age of patients with diabetes mellitus was significantly higher than that of control subjects. There was highly significant difference in the frequency distribution according to marital status, occupation, residency, level of education, economic status in patients and controls groups. It appears that the rate of overweight and obesity is comparable in patient and control group.

Key words: diabetes mellitus; risk factors; prevalence; Iraq; young age

Introduction

Type 2 diabetes mellitus (T2DM) is a complicated disease, the starting age of subjects is as early as 15 years old.2 Diabetes is widespread among people, ages and regions of the sphere due to changes in standard of standards of living, inheritance and environmental factors which all together in the disorder 1. Type 2 diabetes is a serious chronic disease resulting from a complex inheritance environment interaction along with different risk factors such as high BMI and sedentary lifestyle. Type 2 DM and its complications comprise a major worldwide public health trouble, affecting populations in both developed and developing countries with high rates of diabetes related morbidity and mortality. Diabetes mellitus is a clinical syndrome characterized by hyperglycemia caused by absolute or relative shortage of insulin. Lack of insulin affects the metabolism of carbohydrate, protein and fat, and can cause considerable disturbance of water and electrolyte homeostasis; fatality may result from acute metabolic decompensation. Long-standing metabolic derangement is associated with functional and structural changes in numerous organs, predominantly those of the vascular system, which guide to the clinical ‘complications’ of diabetes.

In both of the common types of diabetes, environmental factors interrelate with genetic vulnerability to establish which group develop the clinical syndrome, and its onset. However, the fundamental genes, precipitating environmental factors and path physiology vary considerably between type 1 and type 2 diabetes. The prevalence of diabetes is rising in epidemic magnitude on a global basis. In the USA alone, it has been estimated that there are around 16 million people diagnosed with DM, representing about 6% of the population. Diabetes mellitus is becoming progressively more prevalent in developing countries, possibly due in part to alter in dietary habits, diminished physical activity, and increase BMI.

The worldwide prevalence of DM has risen considerably over the Last two decades, from an estimated 30 million cases in 1985 to 415 million in 2017. Based on current trends, the IDF projects that 642 million individuals will have diabetes by the year 2040. It is estimated that the greater part of patients with diabetes obtain their care from a family doctor. The complexity and chronicity of diabetes presents special challenges for family physicians, whose chief responsibility is the screening and avoidance of diabetes-related complications.
Patient and Method

The type of the design used in this study was a case control. This study was done in the Republic of Iraq, Aldiwanya governorate, in Aldiwanya general teaching hospital. Patient selected randomly throughout their attendance to outpatient clinic of diabetes in the former hospital. The study included period from January 2019 to July 2019. The patient introduce to this study were in the age under 40 years old or has been diagnosed with diabetes below 40 of age, and the study is only for type 2 DM. An arranged questionnaire was prepared by collecting any possible risk factors and Verbal consent was obtained from participant in the study. All diabetic patient with type two who were under the age of 40 or have been diagnosed with DM before that age, we exclude patient that have been diagnosed after the age of 40 years and the patient with type 1 DM. The ethical approval of this study include the following:

- Acceptance of Scientific Committee of Community and Family medicine department in collage of medicine / university of Al-Qadisiyah and acceptance of Committee of Ethical Scientific Researches in the collage
- Verbal consent of individual participated in the study

Results

The Distribution of diabetic patients and control subjects according to age and gender is shown in table 1. Mean age of patients with diabetes mellitus was significantly higher than that of control subjects, 49.93 ±9.95 years versus 34.68 ±12.56 years, respectively ($P <0.001$). No diabetic patient was under 20, 5 (3.1 %) were between 20-29 years, 15 (9.3 %) were between 30-39 years, 51 (31.5 %) were between 40-49 years, 32 (19.8 %) were between 60-69 years and 4 (2.5 %) were between 70-79 years, table 1. The mean age at diagnosis was 36.85 ± 4.48 years with a range of 20-40 years.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Control group n = 149</th>
<th>DM n = 162</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>4 (2.7 %)</td>
<td>0 (0.0 %)</td>
<td></td>
</tr>
<tr>
<td>20-29, n (%)</td>
<td>61 (40.9 %)</td>
<td>5 (3.1 %)</td>
<td></td>
</tr>
<tr>
<td>30-39, n (%)</td>
<td>40 (26.8 %)</td>
<td>15 (9.3 %)</td>
<td></td>
</tr>
<tr>
<td>40-49, n (%)</td>
<td>22 (14.8 %)</td>
<td>51 (31.5 %)</td>
<td></td>
</tr>
<tr>
<td>50-59, n (%)</td>
<td>14 (9.4 %)</td>
<td>55 (34.0 %)</td>
<td></td>
</tr>
<tr>
<td>60-69, n (%)</td>
<td>4 (2.7 %)</td>
<td>32 (19.8 %)</td>
<td></td>
</tr>
<tr>
<td>70-79, n (%)</td>
<td>4 (2.7 %)</td>
<td>4 (2.5 %)</td>
<td></td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>34.68 ±12.56</td>
<td>49.93 ±9.95</td>
<td>$&lt;0.001$ † HS</td>
</tr>
<tr>
<td>Range</td>
<td>17 – 78</td>
<td>23 - 72</td>
<td></td>
</tr>
</tbody>
</table>

| Gender     |                        |           |   |
|------------|------------------------|-----------|
| Male, n (%) | 57 (38.3 %)          | 77 (51.7 %) | 0.099 ¥ NS |
| Female, n (%) | 92 (61.7 %)         | 85 (57.0 %) |   |

$n$: number of cases; SD: standard deviation; †: independent samples t-test; ¥: Chi-square test; HS: highly significant difference at $P \leq 0.01$; NS: not significant at $P \leq 0.05$
There was also highly significant difference in the frequency distribution according to occupation between patients and control groups ($P < 0.001$) in such a way that employee was less frequent in patients than in control group, 32.2 % versus 51.7 %, respectively, whereas the frequency of housewives and retired was more in patients than in control group, 45 % versus 32.9 % and 16.8 % versus 4%, respectively.

In addition, there was highly significant difference in the frequency distribution according to residency between patients and control groups ($P = 0.005$) in such a way that rural residency is more frequent in patients than in control group, 29.5 % versus 14.1 %.

Moreover, there was highly significant difference in the frequency distribution according to level of education between patients and control groups ($P = 0.001$) in such a way that illiterate and primary and secondary levels were more in patients than in control group, 32.9 % and 28.2 % versus 16.8 % and 16.1 %, respectively. Regarding economic status, there was also highly significant difference in the frequency distribution between patients and control groups ($P < 0.001$) in such a way that very poor and poor categories were more frequent in patients than in control group, 13.4 % and 45 % versus 1.3 % and 14.8 %, respectively. The comparison of body mass index (BMI) and waste circumference between diabetic and control groups is shown in table 3. Patients and control subjects were categorized into underweight (< 18.5), normal (18.5 -24.9), Overweight (25-29.9), class I obesity (30-34.9), class II obesity (35-39.9) and class III obesity (≥40 kg/m2), as shown in table 3. It appears that the rate of overweight and obesity is comparable in patient and control group; in addition, there was no significant difference in mean BMI between patients and control groups, 27.02 ±2.83 kg/m2 and 28.01 ±6.85 kg/m2, respectively ($P = 0.091$); however, mean waste circumference was significantly higher in diabetic patients than in control group, 105.85 ±14.26 cm versus 99.21 ±16.62, respectively ($P <0.001$).

### Table 2: Body mass index and waste circumference of diabetic patients and control subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control group n = 149</th>
<th>DM n = 162</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underweight (&lt; 18.5)</td>
<td>1 (0.7 %)</td>
<td>0 (0.0 %)</td>
<td></td>
</tr>
<tr>
<td>Normal (18.5 -24.9)</td>
<td>28 (18.8 %)</td>
<td>34 (22.8 %)</td>
<td></td>
</tr>
<tr>
<td>Overweight (25-29.9)</td>
<td>81 (54.4 %)</td>
<td>99 (66.4 %)</td>
<td></td>
</tr>
<tr>
<td>Class I obesity (30-34.9)</td>
<td>33 (22.1 %)</td>
<td>27 (18.1 %)</td>
<td></td>
</tr>
<tr>
<td>Class II obesity (35-39.9)</td>
<td>4 (2.7 %)</td>
<td>2 (1.3 %)</td>
<td></td>
</tr>
<tr>
<td>Class III obesity (≥40 kg/m2)</td>
<td>2 (1.3 %)</td>
<td>0 (0.0 %)</td>
<td></td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>28.01 ±6.85</td>
<td>27.02 ±2.83</td>
<td>0.091 †</td>
</tr>
<tr>
<td>Range</td>
<td>17 - 78</td>
<td>21 – 35</td>
<td>NS</td>
</tr>
<tr>
<td>Waste circumference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>99.21 ±16.62</td>
<td>105.85 ±14.26</td>
<td>&lt;0.001 †</td>
</tr>
<tr>
<td>Range</td>
<td>23 - 150</td>
<td>78 – 150</td>
<td>HS</td>
</tr>
</tbody>
</table>

$n$: number of cases; SD: standard deviation; †: independent samples t-test; HS: highly significant difference at $P \leq 0.01$; NS: not significant at $P \leq 0.05$
Prevalence rate of smokers was significantly higher in patients with DM in comparison with control subjects, 19.5% versus 12.1%, respectively ($P=0.048$). However, there was no significant difference in prevalence rate of alcoholism between the two groups ($P = 0.171$), as shown in table 3.

Sedentary life style and low activity are significantly more prevalent in patients with diabetes than in control subjects ($P = 0.002$); in addition, with respect to physical activity, bed ridden prevalence was significantly higher in DM patients than in control group, 24.2% versus 2.7%, respectively ($P < 0.001$), as shown in table 4.

### Table 3: Bad habits in diabetic patients and control subjects

| Characteristic | Control group $n = 149$ | DM $n = 162$ | $P$  
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No smoker</td>
<td>120 (80.5%)</td>
<td>111 (74.5%)</td>
<td>0.048 ¥ S</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>11 (7.4%)</td>
<td>22 (14.8%)</td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>18 (12.1%)</td>
<td>29 (19.5%)</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not alcoholic</td>
<td>145 (97.3%)</td>
<td>160 (107.4%)</td>
<td>0.171 ¥ NS</td>
</tr>
<tr>
<td>Ex-Alcoholic</td>
<td>1 (0.7%)</td>
<td>2 (1.3%)</td>
<td></td>
</tr>
<tr>
<td>Alcoholic</td>
<td>3 (2.0%)</td>
<td>0 (0.0%)</td>
<td></td>
</tr>
</tbody>
</table>

$n$: number of cases; ¥: Chi-square test; S: significant difference at $P \leq 0.05$; NS: not significant at $P \leq 0.05$

### Table 4: Life style and physical activity in patients with DM and control subjects

| Characteristic | Control group $n = 149$ | DM $n = 162$ | $P$  
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Life style</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sedentary</td>
<td>4 (2.7%)</td>
<td>20 (13.4%)</td>
<td>0.002 ¥ HS</td>
</tr>
<tr>
<td>Low active</td>
<td>117 (78.5%)</td>
<td>124 (83.2%)</td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>28 (18.8%)</td>
<td>18 (12.1%)</td>
<td></td>
</tr>
<tr>
<td>Physical activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bed ridden</td>
<td>4 (2.7%)</td>
<td>36 (24.2%)</td>
<td>&lt;0.001 ¥ HS</td>
</tr>
<tr>
<td>Moderate</td>
<td>114 (76.5%)</td>
<td>98 (65.8%)</td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>25 (16.8%)</td>
<td>22 (14.8%)</td>
<td></td>
</tr>
<tr>
<td>Very active</td>
<td>6 (4.0%)</td>
<td>6 (4.0%)</td>
<td></td>
</tr>
</tbody>
</table>

$n$: number of cases; ¥: Chi-square test; HS: highly significant difference at $P \leq 0.01$
Discussion

In the current study the mean age of patients with diabetes mellitus was significantly higher than that of control subjects. The mean age at diagnosis was 36.85 ± 4.48 years with a range of 20-40 years, which indicates a significant relationship between DM and aging. This result has been supported by many studies worldwide including study have been done in UK, which prove that type 2 diabetes is more common in the middle-aged and elderly. It affects 10% of the population over 65, and over 70% of all cases of diabetes occur after the age of 50 years. Gender differences arise from sociocultural processes, such as different behaviors of women and men, exposure to specific influences of the environment, different forms of nutrition, life styles or stress, or attitudes towards treatments and prevention. In this study there is no significant relationship between gender and DM, the result have been supported by USA study that found no difference between male and female, study done in Pakistan revealed that there’s no difference in prevalence of diabetes mellitus with the gender. In Iran the prevalence of DM 12.1, with no significant difference between male and female, while the prevalence of diabetes in Saudi Arabia as demonstrated by Al-Nozhal show a higher ratio in females than in males, with 42%, and 37.2%, respectively. There was highly significant difference in the frequency distribution according to marital status between patients and control groups in this study in such a way that the frequency of married is more in diabetic than control which may be attributed to alteration of habitual life style. Study was done throughout Iranian urban population found that marital status was not significantly related to diabetes mellitus. In our study rural residency is more frequent in patients with DM, which has a similar result conducted from a cross sectional study using data from US centers for Disease and Prevention’s (CDC’s)2008 Behavioral Risk Factor Surveillance, has found the increasing prevalence of DM in rural area more than urban that may be attributed to increasing poverty and lower education. Highly significant difference in the frequency distribution according to level of education between patients and control groups. A case-cohort study in eight Western European countries nested in the EPIC study, demonstrates the inequalities in the risk of T2DM in Western European countries, with an inverse relationship between educational level and risk of T2DM that is only partially explained by variations in BMI. Individuals within the middle socio-economic level, who are physically inactive and do not consume large amounts of fruit are at greatest risk of developing type 2 diabetes mellitus as explained by study has been done in Ghana. Individuals within the middle socio-economic level, who are physically inactive and do not consume large amounts of fruit are at greatest risk of developing type 2 diabetes mellitus as explained by study has been done in Ghana as well as in this study.

Conclusion

This study has been focused on the most important risk factors for the development of type 2 diabetes in young age patients and has assimilate other studies conducted throughout the world which puts the BMI, family history, lifestyle, hyperlipidemia, hypertension, and psychological insults in the top of these risks which necessitates the screening for diabetes in earlier ages. Other risk must have further evaluation.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Al-Qadisiyah, College of Medicine, Iraq and all experiments were carried out in accordance with approved guidelines.

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Psychosocial Consequences of Children with Idiopathic Growth Hormone Deficiency in Baghdad

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Abstract

Study aim: to evaluate psychosocial problems of children with growth hormone deficiency.

Methodology: A cross sectional study, of (80) children from both gender, (4 – 15) years diagnosed with idiopathic growth hormone deficiency, attending endocrine clinics in Baghdad city. Consent form taken from children and their guardians to participate in the study. Pediatric Psychosocial Symptoms Inventory (mood, behavioural, somatic, learning, and personality problems) was used to evaluate children psychosocial consequences. SPSS programme version 23 used for data analyses.

Result: children with growth hormone deficiency experience moderate psychosocial problems, especially personality and behavioural problems.

Recommendation: The researchers recommended details psychological assessment for children with growth hormone deficiency during their routine physical examination and provide suitable psychological support for them and their parent.

Key words: psychosocial consequences, growth hormone deficiency

Introduction

Children with growth hormone deficiency usually followed up endocrine clinics for treated their short stature and improve their quality of life (1, 2, 3), they aware about their physical growth and feel with embarrassment about body differences from peers (4). As reported in the pediatric studies that children with health disorders may suffer from different negative psychosocial consequences (5). Those children and their parents influence by children`s short stature, in addition, to the burden of treatment regime (4, 6). In Baghdad a study conducted in 2009 showed the prevalence of short stature only was 18.7%, while with underweight and short stature was 13.5% (7). The prevalence of short stature related to growth hormone deficiency was 1/4000 child and 60-80% of them with idiopathic causes (17). Many previous literature documented adverse effects of the short stature on children`s psychologically and socially (8).

Methods and Materials:

Research design: cross sectional study used, data was collected from first of May tile the first of November 2018.

Setting: study was carried out in outpatient endocrine clinics at two pediatric teaching hospitals and two specialized centres for endocrine disorders in Baghdad city.

Instrument of the study and procedure: Pediatric Psychosocial Symptoms Inventory PEPSI, parents reported scale, Arabic and English version. It has good reliability, developed from two famous pediatric psychosocial scales: strength and difficulties scale and child behavioral checklist scale by Dr. Al -Ayed and Al-Haider in their study about screening of children`s psychosocial problems in Riyadh (9). The approval obtained from the ownerships by electronic mail. PEPSI consists of (38) items, with five subscales: Mood symptoms subscale scored from (0-24), Behavioral

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symptoms subscale scored from (0-27), Learning problems subscale scored from (0-18), Somatic symptoms subscale scored from (0-24), and Personality characteristics subscale scored from (0-21). Rating from 0-3: no symptoms= 0, rarely= 1, occasionally= 2, and frequently= 3. The total score ranged from 0-114 (0-38 = mild, 39-76 = moderate, 77-114 = severe).

**Statistical Analysis**

SPSS programme version 23 was used, frequency, percentage, and mean used in data analysis.

### Results

**Table (1) Pediatric Psychosocial Symptoms Inventory for children with growth hormone deficiency**

<table>
<thead>
<tr>
<th>Levels of scales</th>
<th>Study Group</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>23</td>
<td>28.75</td>
</tr>
<tr>
<td>Moderate</td>
<td>39</td>
<td>48.75</td>
</tr>
<tr>
<td>Severe</td>
<td>18</td>
<td>22.5</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>100.0</td>
</tr>
</tbody>
</table>

This table reflects that (84.75%) of children with growth hormone deficiency experience moderate psychosocial problems when assessed by PEPSI.

**Figure (1) Child’s Age and Gender Distribution**

In these figures, the mean age of children with growth hormone deficiency is (8.41±2.7), most of them (75%) at school age (6-12) years, and mostly are boys (65%).

**Figure (2) Subscales Levels of Pediatric Psychosocial Symptoms Inventory for children with growth hormone deficiency**
Figure (2) shows the details aspects of psychosocial problems (mood, behaviour, learning, somatic, and personality problems) according to Pediatric Psychosocial Symptoms Inventory PEPSI. Generally, children with growth hormone deficiency experience moderate mood problems (46.6%); moderate behavioural problems (60.4%); moderate learning problems (43.3%) moderate somatic problems (47.6%); moderate personality problems (65%).

Discussion

The results of the present study showed that (48.75% and 22.5%) of children with short stature related to growth hormone deficiency experience moderate and severe psychosocial problems respectively. Their physical growth (height) interferes with social competence and body differences from normal peers which caused psychological disturbances. A study about psychosocial problems of children with growth hormone deficiency by using the Visual Analogue Scale for Children, support the present findings that those children showed negative self perception concerning physical appearance 6. Another longitudinal study about terminated treatment of adolescents with growth hormone deficiency showed that, their shortness affects negatively on their psychosocial status and their quality of life 6. Children with growth hormone deficiency suffer from psychosocial disturbances and their psychosocial functioning depending on various factors especially family support and school adaptation 10. Short stature associated with psychosocial distress and emotional deprivation 5. While another study about reviewing the psychosocial consequences of children with short statured that, those children showed negative social experiences but they have not psychosocial problems 5. However some studies mentioned the benefit of growth hormone treatment on their quality of life and self esteem after one year of successful treatment 5.

The present findings showed the aspects of children psychosocial problems according to pediatric psychosocial symptoms inventory PEPSI (learning, somatic, emotional, behavioural, and personality problems), in all aspects of children psychosocial problems children with growth hormone deficiency scored such problems. Mostly they scored behavioural and personality problems (60.4%, 65%). As parent reported their children have mood changes, fight with others, fidgety, tell lies, do not listen to rules, feel down, no hobbies, no friends, spend time alone, depend on others, and seem shy.

Most of children experienced teasing or juvenalization, these negative psychosocial consequences related to their shortness. Those children experienced behavioral and emotional problems, low self esteem. They exposure to bullying and could not adopt with their condition, the psychosocial burdens may also play role in developing short stature among some children 11. Short stature in children can lead to adverse psychosocial impact on those children and affects on their school performance and development of their personality 1. Children with short stature scored behavioural problems as a result of negative social relationships especially with their peers 6. Another study to measured behavioral and social problems of (195) children with short stature compared with normal children found that children with short stature experience higher score of school difficulties and behavioral problems 6. Children with short stature experienced different barriers in their personality development which affects negatively on their school and sport competence 17. Children’s psychosocial problems and school performance are the most concern of their parent 11.

As parent reported children with short stature have low social competence when compared with normal children, because of their unsatisfied appearance which affects on the social relationships and development their personality 10. Those children may face bullying in school or playing with younger children 10. A study in Japan about psychosocial problems of children with growth hormone deficiency by using Child Behaviour Checklist (CBCL) scale, the result of the study showed those children have higher scored than normal children, they also showed high scored on subscale of Child Behaviour Checklist scale such social problems, anxiety and attention 8. Previous studies showed school and psychosocial problems due to their appearance, they experience internalize problems and behavioral problems, and showed low health related quality of life 5. By using child behavior checklist scale for children with short stature, the findings showed those children scored high score of behavioral problems than normal developing children in addition to the somatic, attention, and social problems 14. In a study about reviewing children with short stature reported that those children scored lower in academic functioning, while another American study reported no differences form normal children 5. More than half of children with short stature experienced bullying and showed low quality of life 15.
Children with growth hormone deficiency scored low neuropsychological test and impaired social relation. Those children recorded poor academic level due to effects of social relationships in spite of IQ score comparing with normal children.

Kranzler and his colleague in their study about psychosocial function of children with short stature that, those children scored externalize problems and inadequate social relationships. Children with growth hormone deficiency showed aggressive behavioral problems, attention, social, emotional problems and maladaptive. Prepubertal short children developed more social, internal, and external problems than children in the same age group. Children with short stature experience behavioral problems. Children with short stature scored low self esteem, bullying, juvenilization, and stigmatization because of their shortness. Previous studies reported those children have academic, internal, external, and behavioural problems due to their shortness. Those children have low quality of life. Psychosocial assessment of children with short stature is important in diagnosis and treatment of those children and well being. Some studies reported those children experienced low quality of life, and internal problems. Child health problems affects negatively on their parents.

Early children diagnosis and treatment reduce the risk of psychosocial problems and financial burden of treatment comparing with untreated children, in addition to the psychosocial advantages. Before treatment regimen the psychological profile should be assessed. The psychosocial services should also provide during treatment of children with growth hormone deficiency, those children experience sad feelings because of their shortness and puberty development delayed the psychological assessment of short adult showed some psychological disturbances. The setting and cultural attribute play a role in developing the psychosocial problems associated with certain health problems. The psychological health services will decrease and children well-being improved. Psychosocial improvement is one of the important goals for treated children with short stature.

**Conclusion**

Children with growth hormone deficiency were experience psychosocial problems, especially behavioral and personality problems.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of nursing, University of Baghdad, Iraq and all experiments were carried out in accordance with approved guidelines.

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Prevalence of Hepatitis B and Hepatitis C Viruses in β-thalassemia Major Patient in AD-Diwaya Province, Iraq

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Abstract

Background: transmission of infectious agent is still the most common cause of death and disability related to blood transfusion.

Objective: to estimate the prevalence of HBV and HCV in beta thalassemic major patients in Ad-Diwayah governorate.

Method: The current study is a retrospective cross sectional study involving sample of 80 thalassemic major patients who regularly visit thalassemia center in Ad-diwuryah for treatment, data that required in the study had been collected from patients, their guardians and from the records.

Results: The prevalence rate of HBV was 2.5% (l male and 1 Female ) which was significantly associated with family history of hepatitis ; while the prevalence rate of HCV was 3.8% (1 male and 2 female) which was significantly associated with age and family history of hepatitis.

Key words: prevalence; hepatitis; thalassemia; major.

Introduction

Thalassemia syndromes are characterized by varying degrees of ineffective hematopoiesis and increased hemolysis 1. Clinical syndromes are divided into α- and β – thalassemias, each with varying numbers of their respective globin genes mutated. There is a widen array of genetic defects and a corresponding diversity of clinical syndromes. Most –thalassemias are due to point mutation usually in both of the two β-globin genes (chromosome11), which can affect every step in the pathway of β-globin expression from initiation of transcription to messenger RNA synthesis to translation and post translation modification . A mutation in a single β-globin gene inherited along with triplicated alpha genes also may cause a β-thalassemia syndrome. Autosomal dominant forms of β–thalassemia also occur rarely 1. β-Thalassemia major is caused by mutations that impair beta chain synthesis. Because of unbalanced synthesis of alpha and beta chains, alpha chains precipitate within the cells, resulting in RBC destruction either in the bone marrow or in the spleen . β -Thalassemia major is seen most commonly in individuals of Mediterranean or Asian descent. The clinical severity of the illness varies on the basis of the molecular defect. Signs and symptoms of β-thalassemia major result from the combination of chronic hemolytic disease, decreased or absent production of normal hemoglobin A, and ineffective erythropoiesis. The anemia is severe and leads to growth failure and high output heart failure. Ineffective erythropoiesis causes increased expenditure of energy and expansion of the bone marrow cavities of all bones, leading to osteopenia, pathological fractures, extra medullary erythropoiesis with resultant hepatosplenomegaly, and an increase in the rate of iron absorption. Treatment of β-thalassemia major is based on a hyper transfusion program that corrects the anemia and suppresses the patient’s own ineffective erythropoiesis, limiting the stimulus for increased iron absorption. This suppression permits the bones to heal, decreases metabolic expenditures, increases growth, and limits dietary iron absorption. Splenectomy may reduce the transfusion volume, but it adds to the risk of serious infection. Chelation therapy with deferoxamine or deferasirox should start when laboratory evidence of iron overload (hemochromatosis) is present an before there are clinical signs of iron overload (non immune diabetes mellitus, cirrhosis, heart failure, bronzing of the skin, and multiple endocrine
abnormalities)\(^2\). When it is certain that they require regular transfusion, they should be given washed red cell transfusions at monthly intervals; it is vital that the blood is screened for human immunodeficiency virus (HIV)/acquired immune deficiency syndrome, hepatitis B and C viruses\(^3\). Most death and disability related to blood transfusion worldwide is still caused by the transmission of infectious agents\(^4\). Despite the availability of a highly effective vaccine against hepatitis B, approximately 2 billion people worldwide are infected, 350 million with chronic active infection accounting for (600,000) attributable deaths annually worldwide. Hepatitis B is spread via blood and body fluid contact through heterosexual and homosexual relations, by sharing of needles by infected drug abusers, and by accidental needle sticks in the medical setting. In areas of high disease prevalence (e.g., Southeast Asia, China), transmission is primarily from mother to child during childbirth or in early childhood. The vaccination for hepatitis B uses recombinant DNA, requires three doses on a set schedule, and confers immunity in the majority of recipients. Patients with chronic infection can develop cirrhosis and end-stage liver disease. Hepatitis C affects more than 300 million people worldwide. At least six genotypes and 100 subtypes have been identified. The diagnosis is established with serum testing for HCVRNA antibodies; although an antibody is induced, it is not protective against disease contraction and progression. Transmission occurs via blood or body fluid contamination through IV and intranasal drug use, blood transfusions, and in health care workers (e.g., needle stick or skin disruption with contaminated instrument)\(^5\).

**Patients and Method**

The study has been designed as a retrospective cross-sectional study including a cohort of Iraqi patients having beta thalassemia major on regular transfusion therapy. No limitation for gender or age was proposed. An 80 patients were randomly selected in the study from the population of thalassemic patient regularly visit the thalassemia center in AD-Diwayyah for transfusion therapy (at least once monthly). Any patient had been diagnosed with HBV or HCV infection before starting the first transfusion had been excluded from the study, also family history of HBV or HCV prior to infection of thalassemic persons was one of the exclusion criteria. The study had been done at AD-Diwayyah thalassemia center in AD-Diwayyah governorate in Iraq. The study had been started from first of April 2019 and ended on the third of June 2019. The study had been approved by the committee of ethical approval at Collage of Medicine university of Al-Qadisiyah. Verbal consent had been taken from the patients who included in the study if their age was more than 18 years or from their guardians if they were younger than 18 years old. The questionnaire had been designed to involve sociodemographic informations of patients included in the study like gender, age, residency; if it was rural or urban area and occupation. Socioeconomic state also was part of the questionnaire, the patients classified as high, moderate and low socioeconomic state; taking in consideration their level of education, income, residency, number of family member comparing with number of rooms and the house size, also if the house was renting or their own.

**Results**

**Distribution of patients with beta thalassemia major according to age**

The frequency distribution of patients with beta thalassemia major according to age is shown in table 4.1. Patients less than 10 years old accounted for 29 (36.2 %), patients between 10 to 19 years old accounted for 38 (47.5 %), patients between 20 to 29 years old accounted for 8 (10.0 %) and patients between 30 to 39 years accounted for 5 (6.2 %). The mean age of all participants was 13.38 ±8.26 years with a range of 2 to 39 years, table 1. Majority of patients were between 10 to 19 years and <10 years of age, as shown in figure 1.

**Table 1: Distribution of patients with beta thalassemia major according to age**

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10 years, n (%)</td>
<td>29 (36.2)</td>
</tr>
<tr>
<td>10-19 years, n (%)</td>
<td>38 (47.5)</td>
</tr>
<tr>
<td>20-29 years, n (%)</td>
<td>8 (10.0)</td>
</tr>
<tr>
<td>30-39 years, n (%)</td>
<td>5 (6.2)</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>13.38 ±8.26</td>
</tr>
<tr>
<td>Range</td>
<td>2 – 39</td>
</tr>
</tbody>
</table>

\(n\): number of cases; \(SD\): standard deviation
Distribution of patients with beta thalassemia major according to gender

The frequency distribution of patients with beta thalassemia major according to gender is shown in figure 2. Male patients accounted for 41 out of 80 (51.2 %), whereas, female patients accounted for 39 out of 40 (48.8 %). The male to female ratio was 1.05:1.

Distribution of patients with beta thalassemia major according to residency

Distribution of patients with beta thalassemia major according to residency is shown in table 2. Patients from urban areas accounted for 27 out of 80 (33.8 %) and patients from rural areas accounted for 53 out of 80 (66.2 %), table 2.

Table 2: Distribution of patients with beta thalassemia major according to residency

<table>
<thead>
<tr>
<th>Residency</th>
<th>*N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban</td>
<td>27</td>
<td>33.8</td>
</tr>
<tr>
<td>Rural</td>
<td>53</td>
<td>66.2</td>
</tr>
</tbody>
</table>

*n: number of cases

The prevalence rate of hepatitis B and C viral infection in patients with beta thalassemia major

The prevalence rate of hepatitis B and C viral infection in patients with beta thalassemia major is shown in table 3. Two patients out of 80 were infected with HBV accounting for 2.5 %, while 3 patients out of 80 were infected with HCV accounting for 3.8 %, table 3.

Table 3: Hepatitis B virus and hepatitis C virus infection, positive family history of HBV and HCV and history of hepatitis vaccination

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N*</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td>HCV</td>
<td>3</td>
<td>3.8</td>
</tr>
<tr>
<td>Family history of HBV</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td>Family history of HCV</td>
<td>2</td>
<td>2.5</td>
</tr>
<tr>
<td>Vaccine</td>
<td>80</td>
<td>100.0</td>
</tr>
</tbody>
</table>

*n: number of case

Correlation between HBV or HCV infection and age of patients with beta thalassemia major

Mean age of patients with HBV were older than patients who were free of HBV infection, 20.00 ±2.83 years versus 13.21 ±8.29 years, respectively; however, the difference did not reach statistical significance (P = 0.253). Mean age of patients with HCV were older than patients who were free of HCV infection, 24.33 ±12.74 years versus 12.95±7.86 years, respectively; and the difference was statistically significant (P = 0.018), as shown in table 4.

Table 4: Correlation between HBV or HCV infection and age of patients with beta thalassemia major

| Hepatitis virus | n  | Mean age (years) | SD  | P       |
|-----------------|--|--|------------------|-----|---------|
| HBV  Positive   | 2  | 20.00            | 2.83| 0.253 † |
|                | 78 | 13.21            | 8.29| NS      |
| HCV  Positive   | 3  | 24.33            | 12.74| 0.018 † |
|                | 77 | 12.95            | 7.86| S       |

*n: number of cases; SD: standard deviation; †: Mann Whitney U test; NS: not significant at

Discussion

The current study had been designated in order to highlight the prevalence of HBV and HCV infection beta thalasemic patients in Ad-Diwanyah province since those patient need multi transfusion therapy for the rest of their lives, the fact that make them at higher risk to have blood transfusion transmitted infections, including HBV and HCV, and these infections can be transmitted to healthy persons by many routes other than blood transfusion and causing dangerous morbidity that reach hepatocellular carcinoma or even death. Also there is no other published study concerning about this topic in the mentioned area. Patients with beta thalassemia major had been targeted in the current study because those
patients need blood transfusion in a very early life time mostly in the first year of life so, if those patients had been infected with HBV or HCV, that would be most likely due to transfusion of contaminated blood.

Regarding age distribution in thalassemic patients who involved in the study, it was ranging from 2 to 39 years old with 83.7% of them less than 20 years old which could be due to high fatality rate of thalassemic patients comparing with general population .This result had been agreed by many other studies in the vicinity like Kamal Dumaidi and co-worker study about prevalence of hepatitis C and B viruses among patients with B thalassemia in Palestine which reveal that (75%) of patients involved in the study were aging 24 years and less 8. The mean of age in the current study was 13.38±8.26 year , this result was near but less than the mean age in the studies that done in the vicinity , as in the study that had been done in Iran by silos Mohammadi and Mazaher Khodabandehloo about the prevalence of HCV antibodies among Beta thalassemia major patients which revealed that the mean age was 18±8.05 years 7. Male to female ratio was 1.05:1 since the inheritanc of thalassemia is autosomal which was agreed by Al-Naamani study in Oman 8. 66.2 % of patients who included in the study were living in rural areas. Regarding occupations of patients who enrolled in the current study; most of patients were either so small to attend school , students or having no job apart from single patient who was a nurse. Most of patients involved in the current study was classified as low or moderate socioeconomic state (87.5%) and the other 12.5% was classified as high socioeconomic state. 85% of patients’ parents were relative since most of them live in rural areas in which there is high rate of marriage between relatives. The rate of blood transfusion was ranging from 1 to 4 times per month patients with small age and patients who had splenectomy usually need transfusion 1 - 2 times per month while patients who didn’t have splenectomy and reaching adulthood or teenage , they usually needs transfusion 3-4 times per month. The prevalence rate of hepatitis B virus infection in patients participating in this study was 2.5% (two patients out of 80) which was less than the result of similar study done by Widad Yazaji in Syria which is a neighboring country, the study involved 159 patients, the study reveal that 13.2% of patients were seropositive for hepatitis B infection (21 patients out of 159) 9. The prevalence rate of HCV infection in the current study was 3.8% (3 patients out of 80) which was slightly higher than the prevalence rate of HBV infection which might be due to the availability of HB vaccine in the national vaccine program while there is no vaccine for HCV till the current day in the entire world. The prevalence rate of HCV infection in patients with thalassaemia in Zabol city of Iran; another neighboring country, was 8.5% 12 which was higher than the current study which might be due to higher prevalence in general population in Iran (0.5%) 13 comparing with Ad-Diwanyah population (< 0.3%) 14. Regarding to relation of gender with HBV and HCV infection, there was no significant relation between them, this result going with study of Ansari et al 16. In the current study, there is no association between residency and infection with HBV and HCV. Also , no significant association between socioeconomic state and infection with HBV and HCV. This result had been agreed by Ghufranud Din’s et al study in Pakistan 17.

Conclusions

Prevalence of HBV and HCV infection is more in multitransfused beta thalassemia major patient than in the general population. Measures taken in the blood preparation and checking still not enough to prevent infections transmitted by blood transfusion. Risk of having HBV and HCV infection in multitransfused patients increase with progression of age. There was highly significant association personal and family history of HBV and HCV infection.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Medicine/ University of Al-Qadisiyah and all experiments were carried out in accordance with approved guidelines.

References

Deposition of TaN Film on Commercial Pure Titanium Disk by Modified Reactive Plasma Sputtering Technique

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Abstract

Background: The new trend of implants is to find materials which accelerate bone formation at bone implant interface and improve Osseo integration to provide immediate or early loading after placement and eliminate waiting period which is uncomfortable and disturbs patients. Titanium as an implant material still need some improvement of surface properties physically and chemically. Tantalum which is gaining more attention as a new metallic biomaterial. Coating layer over implant is an important way for improvement of surface properties of titanium. Plasma used for surface modification, has several advantages such as changing surface topography, increasing surface roughness and in increasing the wettability of the surface.

Aim of study: To evaluate the effect of TaN coating by modified plasma sputtering technique of commercially pure titanium disk on wettability, surface roughness, surface chemical composition in comparison to non-coated surface.

Materials and methods: Two groups were tested in this study which include non-coated commercial pure titanium disks and coated commercial pure titanium with TaN. Modified reactive plasma sputtering apparatus was used to coat CpTi with TaN at 4, 6 & 8 h. Surface characterization by x-ray diffraction (XRD) analysis, scanning electron microscope (SEM), energy dispersive spectroscopy (EDS), contact angle measurement, were carried out for coated and uncoated disks.

Results: The result of coating specimen with TaN at times (4, 6 & 8) h showed that 8 h coating time was the best time. And this was according to the results of X-ray diffraction analysis which show a new peak formation of TaN coated CpTi disk which was not present in non-coated CpTi disk. The results of wettability test for CpTi disk coated with TaN disk was more than wettability of non-coated CpTi disk. Surface Roughness was more and better distributed in CpTi coated with TaN disk than non-coated one which appear clearly in electron microscope.

Keywords: TaN film, pure titanium disk, modified reactive plasma sputtering technique

Introduction

Engineering and surface modification of dental implant material become much advanced than it was earlier 1. Titanium shows a favorable combination of intrinsic properties for the fabrication of dental implants such as low specific weight, high strength to weight ratio, low modulus of elasticity, very high corrosion resistance, easy surface coating and excellent general biocompatibility 2 enhancing the osseointegration of CpTi results in reducing the non-functional time period of the implant, increase its applicability in alveolar bone with low quality, cause minimum discomfort to patient, and minimize the failure rates. 3. Surface coating was one of the methods used in accelerating the Osseointegration process and this by increasing roughness and change surface properties physically and chemically. Chemically and physically reactive plasma discharges are widely used to modify the surface properties of materials. 5. The stable chemical element Tantalum (Ta) can exist in the surface layers of base materials. Ta is one of the promising materials used in medical and dental fields 6. The stable TAN protective film can provide better
corrosion resistance and bioactive property than that of TiO film. The Ta components offer a low modulus of elasticity, high surface frictional characteristics, and excellent Osseo integration properties (i.e. Bioactivity and biocompatibility). Surface-coated implants are reported to have less failure and can support heavier and more dynamic forces surface coating with TaN by using plasma sputtering techniques was applied in this study on Cp Ti disk. In dental field especially in dental implant, plasma coating constitutes a simple, dry technique, which does not harm the environment, of low cost and does not comprise the intrinsic properties of the biomaterial, affecting only its surface. Many types of gases can be used in such technique to modify surface properties physically and chemically. Aim of our study to coat CpTi with TAN by using coat plasma sputtering technique.

Material and Method

Sample preparation

Titanium disks were prepared (10×5mm) diameter and thickness respectively which were cut from Cp Ti rod by using Bench Nibbling machine (TAURUS 7000-W6 CNC, Italy). All specimens were abraded successively using SiC grinding paper with different grits started from 80 grit, and continued by 120, 230, 400, 600, 800 and 1000 grit to get flat and scratch free surface. These disks were polished until a smooth and mirror polished surface was obtained. After that all specimens cleaned by using ultrasonic cleaning device

Surface coating with TaN:

The procedure of sputtering was performed by using modify Dc glow discharge reactive plasma sputtering system. The total number of samples were 10 disk which divided as 1 uncoated and 9 coated disk. These coated disks were divided into 3 groups according to the time of coating (4, 6, &8) h. The procedure of sputtering started with placing the clean and polished disks on the center of anode base. Then evacuating process of chamber to high vacuum (~1x10^-5 mbar) by using high vacuum system which consist of rotary and turbo molecular vacuum pumps to ensure the complete removing of the heavy gases like hydrocarbons. After that Power supply negatively charged voltage of 3.5 kV applied. For sputtering process, the voltage was gradually applied using variac until the required energy achieved (applied voltage and current), The pressure was 2x10^-2 to 7x10^-2 mbar and this pressure was achieved by feeding the bombardment and reactive gases. The appropriate voltage and amber were adjusted precisely by regulator until intended sputtering glow (purple color, which standardized for each gas) is achieved. One of the most important modification done to convert normal plasma system to sputtering plasma system include replacement of electrode position, so the cathode placed in upper part and anode electrode placed in lower part of chamber. A Target (cathode) and anode stainless disk. The cathode faced the anode with 7 cm distance between them, which provides electric field for the gas to be discharged. The electrical electrodes and the associated dc-power supply of 5kV. The bottom of the stainless steel disk cathode electrode are covered with tantalum sheet which regarded as target. The clean and polished samples were placed on the anode in the center of base which regarded as substrate. All the samples were cleaned by argon plasma sputtering for 15 min prior to the TaN coating process by applying a bios dc voltage of 100 Von the anode. Parameters used for reactive plasma sputtering procedure: argon was used as bombardment gas and nitrogen as reactive gas. The process was carried out at various sputtering times (namely 4, 6 and 8 hours). The reactive gas nitrogen was introduced into the evacuated chamber and the flow rate was adjusted until the pressure was stabilized to the required pressure (1×10^-2 mbar), then the argon gas introduced to the chamber until the sputtering pressure of 5×10^-2 mbar achieved. After the sputtering process completed, the samples were kept until ambient temperature in the vacuum chamber was reached.

Phase Analysis by using X-Ray Diffraction

Surface analysis for Phase’s distribution was performed for non-coated CpTi, and CpTi coated with TaN. It done by using X-ray Diffraction Facilities (SHIMADZu 6000, Japan) using Cu Kα radiation. XRD analysis were performed at room temperature in the 2θ range from 30˚- 80˚ with a 0.05˚ step and counting time of 5 secs per step. The indexing of the data and the diffraction peaks were identified according to the powder diffraction files. (PDF), received from ICDD (Intimations’ Center for Diffraction Data).

Wettability test

In this test, non-coated and coated disk (10×5) mm diameter and thickness respectively were used. An equal amounts of normal saline (0.25ml) from graduated
container dropped on each disk. Then after 20 second from putting the drop on disk, surface measurement for the angle formed between titanium disk surface and drop of normal saline done by taking a picture obtained from a digital camera.  

**Scanning electron microscope examination**

All tested group were examined by using SEM (JEOL-JSM-5600). Samples were prepared as cross section and then mounted in double face metal tape (electrically conductive) to make the sample holder electrically conductive before entering the samples into the SEM Chamber. VEGA3 TESCAN, SEM HV: 20 KV, SEM MAG:7.50kx, VIEW FIELD:27.7

**Energy dispersive spectroscopy analysis**

Chemical structures and relative concentrations for non-coated CpTi and coated CpTi with TAN discs were assessed via energy dispersive spectroscopy (EDS). EDS use of the X-ray spectrum radiate a solid sample with a focused beam of electrons to obtain a localized chemical analysis. All elements from atomic number 4 (Be) to 92 (U) can be detected in principle. Qualitative analysis involves the identification of the elements in the spectrum and is fairly straightforward owing to the simplicity of X-ray spectra. Quantitative analysis determination of the concentrations of the elements.

**Atomic force microscopy examination**

Atomic force microscope able to detect both conductive and nonconductive surfaces on the atomic scale. The AFM relied on the scanning technique and provided a high-resolution 3D image from the surface of the sample. A sharp tip at the end of the cantilever is in contact with the surface of the development and the sample displaced with piezoelectric scanners. The force on the tip causes deflection to measure with tunneling capacitive or optical detectors such as interferometer laser in this technique, the standard pressure applied to the joint is zero (to prevent any surface deformation).

**Result**

**Surface characterization by using X-Ray Diffraction.**

**A: Before Heat treatment:**

XRD patterns of the non-coated Cp Ti specimen and TaN coated by modified reactive plasma sputtering after different sputtering time (namely 4, 6 and 8 hours) are demonstrated in Figure (1). and these data were indexed according to the Powder Diffraction Files (PDF) for the hexagonal αTi (JCPDS-ICDD file # 44-1294), TaN (JCPDS-ICDD file # 25-0922, 33-1391and 33-1390), and TaN (JCPDS-ICDD file # 34-0977). The diffraction peaks of the uncoated CpTi were found to be corresponding to (100), (002), (101), (102), (110), (200), (112) and (201) α-Ti at 2θ values 35.00°, 38.30°, 40.05°, 52.9°, 62.8°, 70.6°, 76.25° and 77.35° respectively. The patterns of the plasma sputtered specimen for 4 and 6 hours show wide peak in the range of 2θ 20-30° which seems to be due to the formation of not fully crystalline TaN, whereas the pattern of the specimen sputtered for 8 hours show clear and prominent TaN peaks corresponding to the reflections (111) at 2Θ 35.14 and (200) at 2Θ 41.24°. Also its very clear there are a shift in the 2θ position of the CpTi reflections towards the higher 2θ indicting the change in titanium crystal cell volume.

**B: After Heat treatment:**

The CpTi specimen’s coated with TaN for sputtering time 8 hours before and after heat treatment are shown in Figures (2). The pattern of the heat treated specimen show prominent new peaks of 111 TaN at 2θo 36.00 and developed one assigned as 200 TaN at 2θo 41.24. also The XRD pattern of the heat treated specimen shows the formation of 110 of tantalum peak TaN at 2θ 27.23.
Surface characterization using Wettability test

From the result of wettability test, 8 hr. coated disk with TaN give more hydrophilic surface feature with contact angle formed less than non-coated disk as shown in Figure (3).

<table>
<thead>
<tr>
<th>Material/Time</th>
<th>Cp Ti Contact Angle</th>
<th>TAN Contact Angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Non coated</td>
<td>60°</td>
<td></td>
</tr>
<tr>
<td>B: 4h coating</td>
<td>75°</td>
<td></td>
</tr>
<tr>
<td>C: 6h coating</td>
<td>65°</td>
<td></td>
</tr>
<tr>
<td>D: 8h coating</td>
<td>55°</td>
<td></td>
</tr>
</tbody>
</table>

Fig (3) Wettability test for CpTi and experimental groups at 4, 6, and 8 hr.
Surface characterization using SEM 3.3

A: Topographic. The scanning electron microscopy images of CpTi disk before coating in (figure 4a) revealed a relatively flat and smooth surface. While the surface morphology of CpTi plasma sputtering samples with TaN for 8 h showed a fully arranged nanochips and uniformly distributed in (figure 4b).

![SEM images](image)

A: CpTi disk B: CpTi disk coated with TaN

Figure (4): SEM A/CpTi disk without coating B/CpTi disk coated with TaN for 8 hr.

B: chemical composition by EDS

![EDS images](image)

Regarding the analysis of the chemical structure of CpTi figure (5, A) show two titanium peaks with these values (kα 4.512 and lα 0.452), these values represent alpha phase of titanium. For coated disk with TaN, there were five peaks, two peaks for titanium which represent alpha phase, two tantalum peaks and one peak of nitrogen with following values percentage (kα 4.512 and lα 0.452) (lα 8.146 and mα 1.712) (kα 0.392) respectively as shown in figure (5, b). Elemental composition of tested group as follow in (fig a) titanium was 100%, and in (fig b) percentage of titanium was 3%, tantalum was 22% and nitrogen was 75%.

A: CpTi disk B: CpTi disk coated with TaN

Fig (5): EDS A/ CpTi disk without coating B/CpTi disk coated with TAN for 8 hr.

Discussion

The new trend in implant research is to increase and accelerate the osseointegration of Cp Ti. One of the methods used in accelerating the osseointegration process is to modify the surface properties of the implant. Chemically and physically reactive plasma is widely used to modify the surface properties of materials. In this research, tantalum is a material with specific properties which is used as a coat material with nitrogen gas to develop TaN surface on CpTi disk by using modified plasma sputtering technique which has several advantages such as changing surface topography, increasing surface roughness, wettability of the surface. Reactive plasma coating constitutes a simple, dry technique, which does not harm the environment, does
not comprise the intrinsic properties of the biomaterial.

The results of X-ray diffraction analysis revealed a new peaks formation towards the 2θ direction in coated disk as a result of deposition of TaN material on the surface of CpTi, these new peaks were more prominent after heat treatment in coated disk as evidence to the more crystallinity of TaN. Surface properties and roughness of uncoated CpTi and TaN coated disk for 8 h were examined by Scanning electron microscope, showed fully arranged nanochips with uniform shape in coated layers, this might be due to difference of chemical composition of coated layer of non-coated and TaN disk, this shape of nanochips due to the deposition of TaN on the surface of CpTi. Contact angle of fluid drop in 8 hr. CpTi coated disk with TaN less than non-coated CpTi disk, which mean the coated disk more hydrophilic than non-coated disk. This may be due to more affinity of tantalum and nitrogen to fluid.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of dentistry. University of Karbala and all experiments were carried out in accordance with approved guidelines.

References


Influence of Carcinogenic Substance (7, 12 Dimethylbenz [a] Anthracene (DMBA) on Tissue, Hematology Character and Enzyme Activity in Rat

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Abstract

This study designed to investigate the effects of 7,12 dimethylbenz [a] anthracene (DMBA)on some blood parameters and hepatic histopathology in rats and antioxidant enzyme. twenty female Wistar albino rats, weighing 180–200 g, were randomly divided into two group 1- DMBA group (positive group) who received 20 mg DMBA/kg body weight/ (single Douse) 2-Control group (negative group) doesn’t receive any item. The animals in these groups were sacrificed at the end of the 90 days:The histological structure of the liver tissues in the control group was normal. the liver exhibited hydropic degeneration and coagulation necrosis in hepatocytes, severe dilation in the sinusoids, congestion in the central and portal regions , DMBA groups, was degenerative and necrotic changes were detected.MDA levels increased in the DMBA group, compared to the control group, Antioxidant activity CAT, SOD and GPX, the results showed increased enzyme activity in positive control compared with negative groups , ,the hematological parameter was increase in WBC, PLT, GRA, and LYM and decrease in RBC value .

Keywords: 7,12-dimethylbenz[a]anthracene (DMBA); Blood parameters; Antioxidant enzyme ; MDA ; Rat.

Introduction

Malignant neoplasm, is that diseases in which a crowd of cells illustrate abnormal growth, invasion and sometimes called metastasis . This disease begins in the moment that cells in body start to grow without control . More than 100 kinds of neoplasm are identified . The stimulation of tumors is named carcinogen, that have the capacity to change DNA in behavior that are inherited by daughter cells which called mutagens. Most of these tumors are associated to ecological, life, or expected behaviors . The statement “ecological”, as used by cancer investigators, means everything outside the body that reacts with humans . On the other hand some chemicals, like benzene, ketones, vinyl chloride, ethylene bromide, and dichlorodiphenyl-trichloroethane (DDT), known as carcinogens . When multiple factors are accumulated, altered genes and carcinogens change a normal cell into a cancerous one. Carcinogens which promote cancer have various precursors , exogenous and endogenous factor lead to cause Cancer development such as nutritional habits (food preservation and preparation), socio-economic status, way of life, physical agents (ionising and non-ionising radiation), chemical compounds (natural and synthetic) and biological agents (Helicobacter pylori, Epstein Barr virus, human T lym-phototropic viruses I and II, human papilloma virus and thehepatitis B virus .

Material and Method

Experimental animals and Tumor induction in rats:

Twenty female of Wistar albino rats and aged between (7-8) week were weighing between 150 – 250 g used in this study. All they were kept in ventilated cages, with temperature of 25±2C°. A 12:12 h light:dark cycle is regulated for the animals. Balanced rodent food and water is provided. The rats were randomly assigned to 2 groups, every group of 10 rats.

The following groups:
Group 1: Taking one dose of DMBA (positive control) 20 mg DMBA/kg body weight (single dose)

Group 2: Did not received any treatment (negative control).

**DMBA administration:** Breast cancer that induced by 7, 12-

Dimethylbenz (a) anthracene (DMBA). One dose of DMBA dissolved within corn oil and given orally to each groups with the syringe and needles. DMBA was taken from Sigma Aldrich and melted in corn oil. The concentration of the mixture was 20 mg DMBA per 1 ml corn oil for each rat.

**Preparation of tissue sample**

The liver was exteriorized and excised. All samples in the same time fixed in 10% formaldehyde. And then they processed in known method, and placed in paraffin for histopathological testing for liver. A scoring system (of no abnormalities, mild, moderate, severe) was used to classify the liver changes according to the severity of the damage and extent of histological changes. The histological sections were evaluated by a pathologist without prior knowledge of the treatment given to the animals.

Blood parameters were determined in whole blood by the applying rat method of veterinary practice with a blood cell counter (Abocus Junior Vet-5, Austria). Measurements of biochemical parameters were made with a Modular PP autoanalyzer (Mindray BS800, China).

**Result**

Histopathological findings: In the DMBA group, the livers exhibited dilatation of the sinusoids, cholangiohepatitis in the portal region, and congestion in the sinusoidal and portal regions (Figure 1A). The control group showed normal histological structure in the liver tissue (Figure 1B).

Hematological parameters also change in negative group compared of positive group Table 1, in positive group: white blood cell (WBC), LYM, MON, GRA and PLT while other parameter such as RBC was decrease. The state of free radicals and antioxidants is given in Table 2. As seen in Table 2, the levels of MDA increased in liver groups in compare with control group, also SOD, GSH-Px, CAT, and GSH values decreased.

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameter</th>
<th>Negative control</th>
<th>Positive control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>WBC (10³ /mm³)</td>
<td>8.2</td>
<td>15.1</td>
</tr>
<tr>
<td>2</td>
<td>RBC (10³ /mm³)</td>
<td>8.8</td>
<td>1.4</td>
</tr>
<tr>
<td>3</td>
<td>LYM %</td>
<td>67%</td>
<td>78.5 %</td>
</tr>
<tr>
<td>4</td>
<td>MON %</td>
<td>1</td>
<td>0.9</td>
</tr>
<tr>
<td>5</td>
<td>GRA %</td>
<td>18.8 %</td>
<td>94 %</td>
</tr>
<tr>
<td>6</td>
<td>MCV (fl)</td>
<td>56.6</td>
<td>58.7</td>
</tr>
<tr>
<td>7</td>
<td>MCH (Pg)</td>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td>8</td>
<td>MCHC (g/dl)</td>
<td>32.3</td>
<td>32.8</td>
</tr>
<tr>
<td>9</td>
<td>PLT (10⁵ /mm³)</td>
<td>338</td>
<td>737</td>
</tr>
</tbody>
</table>
Table 2. Some biochemical parameters in the groups (values are mean n=4)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Conc. of GPx(IU/mg)</th>
<th>Conc. Of SOD(IU/mg)</th>
<th>Conc. of CAT(IU/mg)</th>
<th>Conc. of MDA(nmol/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative group</td>
<td>127</td>
<td>458</td>
<td>122</td>
<td>134</td>
</tr>
<tr>
<td>Positive group</td>
<td>312.3</td>
<td>567</td>
<td>187</td>
<td>267</td>
</tr>
</tbody>
</table>

Discussion

7, 12-dimethylbenz (α) anthracene (DMBA) is a famous carcinogen and immunosuppressor used in rodent model to learn tumor. DMBA is reported to make mutations by creation DNA adducts, while, it is a well-known skin carcinogen, however, lots of researchers have reported the harmful result of DMBA in liver. Liver is the main site of metabolism and is frequently prone to injury by xenobiotics. Obviously, liver cancer is the second most general source of cancer deaths universal.

A number of hematological and haematochemical parameters were altered when treated with DMBA and establish that DMBA caused hepatocellular carcinoma. New studies showed that DMBA-induced skin, oral, mammary and ovarian tumors.

The carcinogenic and mutagenic effect of DMBA needs to its metabolic activation by mixed gathering oxidases. The hydroxylation of DMBA at 7-methyl collection is a critical step towards its carcinogenesis. Additional metabolism of DMBA leads to development of a large range of metabolites with changeable toxicity. Along with these, trans-3,4-dihydrodiol-1,2-epoxide is the carcinogenic result of DMBA. The metabolic products of DMBA, when present within body, hampers ROS-antioxidant balance by overproduction of free radicals and the body go round reacts by modulating activities of antioxidant enzymes to control the destructive effects of an enlarged ROS.

Hematological and biochemical parameters may be affected by a variety of factors such as race, age, gender, pregnancy, lactation, muscular activity, area, season, environmental heat, maintenance, and nutrition.

A significant increase in the GPX, CAT, SOD level in rat treated with DMBA only was reported. On the other hand, find MDA substance increase significantly with rat treated with DMBA only.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Warith Al-anbiya’a, Iraq, Iraq and all experiments were carried out in accordance with approved guidelines.

References


Immunohistochemical Expression of CD68, P53 and Bcl2 in Thyroid Tumors

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Abstract

Background/aim: The aim of this study was to determine the expression of CD68, P53 and Bcl2 in thyroid tumors and correlation between them.

Materials and Method: The expression of CD68, P53 and Bcl2 was examined by immunohistochemistry on paraffin-embedded tissues obtained from patients with benign and malignant tumors between 2010 and 2015. Thirty three malignant thyroid tumors of variable subtypes and 10 benign tumors. The extents of staining and intensity were scored semi quantitatively.

Results: CD68 have shown strong positive score expression in (28.9%) in PTC while score was found in (18.5%). The higher percentage of strong weak positive score expression of P53 was in PTC (6.1%) Followed by (3.03%) strong score in FTC, (3.03%) moderate score in benign and (3.03%) in MTC. The expression of Bcl2 was higher in benign tumors (20%) than in malignant tumors were PTC (9.1%) followed by FTC (3.03%), the strong positive expression seen in MTC (9.1).

Keywords: Thyroid tumors, Immunohistochemistry, CD68, P53, Bcl2.

Introduction

The commonest type of endocrine malignancy is thyroid cancer, although it is rare. Thyroid cancer accounting for about 1.5% of all newly diagnosed cancer in the United States, its occurrence being steadily increasing worldwide in the last three decades. It is categorized into three main histological types: differentiated including “papillary, follicular and hurthle» «medullary» and «anaplastic» (aggressive undifferentiated tumor). Papillary thyroid carcinoma (PTC) represents 80.3% of malignant endocrine tumors and 65% of malignant thyroid tumors. According to Iraqi cancer registry, it ranks the eight cancers from commonest ten cancers in general and the second cancer in female (Iraqi cancer registry, 2019). Combined with there are different cellular and molecular factors that plays a role in development and progression of thyroid cancer. One of the main cellular constituents in the stromal of many cancers is “tumor-associated macrophages” (TAM). Advanced staging and poor prognosis in many human cancers including thyroid cancers related to tumorigenic role of “TAM”. TAMs have an essential role in tumor advancement at different levels, including stimulating genetic instability, nurturing cancer stem cells pavement the way to metastasis, taming defensive adaptive immunity. Transformations in the p53 gene are the most widely recognized in human cancer and they express to the most frequent genetic changes in malignant transformation. P53 protein assumes an essential role in the regulation of the cell cycle. Wild sort p53 protein is capable of inhibiting cell proliferation and transformation and it has been observed to be latent in tumor cells. This gene mutation is the most prevalent genetic alterations in human tumors and has been found in over 15% of thyroid neoplasm. The p53 gene mutations are promoting more aggressive cancers. Bcl2 protein is a modular of programmed cell death and is involved in both lymphoid and epithelial malignancies. The reporting of bcl2 protein expression in thyroid cancer has been sporadic and some have shown a down regulation of bcl2 in papillary carcinomas.

The present study aimed to evaluate level of CD68, P53 and Bcl2 expression in both malignant and benign thyroid...
tumors, and to correlate the results with the variable clinicopathological parameters (taking in respect the age and sex of the patients combined with tumor types histopathological).

**Materials and Method**

Our study involved paraffin-embedded tissue blocks samples of 43 thyroid tumors patients, of which 33 thyroid carcinomas and 10 benign as control group. All these samples were collected from the Pathology Department of Teaching Laboratories /Medical city and Central Public Health Lab between the years 2010-2015. All Hematoxylin and Eosin stained tissue sections were reviewed by pathologist. Immunohistochemistry study was prepared on serial sections and the antibodies panel that has been used is shown in the table below (table 1).

**Table 1: Antibodies used for immunohistochemical study**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Code</th>
<th>Clone</th>
<th>Antigen retrieval</th>
<th>Dilution</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD68</td>
<td>M 0814</td>
<td>KP-1</td>
<td>pH.9</td>
<td>1:50-1:100</td>
<td>Dako/Denmark</td>
</tr>
<tr>
<td>P53</td>
<td>M 7001</td>
<td>DO-7</td>
<td>pH.9</td>
<td>1:25-1:50</td>
<td>Dako/Denmark</td>
</tr>
<tr>
<td>Bcl2</td>
<td>M 0887</td>
<td>124 clone</td>
<td>pH.9</td>
<td>1:50-1:100</td>
<td>Dako/Denmark</td>
</tr>
</tbody>
</table>

CD68, P53 and Bcl2 immunohistochemical staining.

Immunohistochemistry: Four µm sections were cut from formalin-fixed, paraffin-embedded tissue blocks and placed on polylysine-coated glass slides. After overnight packing at 65°C, tissue sections were deparaffinized in xylene and rehydrated in descending grades of alcohol. Antigen retrieval was performed in a water bath for 15 min in 95 °C citrate buffer pH 9.0 for CD68, P53 and Bcl2. Endogenous peroxidase action was blocked by 3% hydrogen peroxide. The sections were incubated at room temperature for 1 hour with primary monoclonal antibodies.

**Scoring of immunohistochemistry:** The stained slides were observed microscopically by histopathologist using the p53, Bcl2 and semiquantitative criteria: 0= negative; 1+ =<33% area of positive staining; 2+ =34-66% area of positive staining; 3+ = >67% area of positive staining. Positive staining was also graded on intensity, 0 to 2+. A combined score of 0 to 6 was allocated. Tumors assumed a score 0 to 1 were classified as negative; those given a score of 2 were ordered as weakly positive; a score of 3-4 was regarded as moderately positive; and a score of 5-6 was considered strongly positive.  

The proportion of CD68 cells in each tumor on each slide was assessed after staining. Based on a median value of 25%, weak (+) and strong positive (++) staining were defined as <25% and ≥ 25%, respectively.

**Statistical Analysis**

Data were analyzed by one -way analysis variance (ANOVA) followed by Fisher’s test for multiple comparison, using Statview version 5.0. Differences were considers “significant when p<0.05”. Reversion analysis was done by analysis of covariance (ANCOVA) likewise by Statview version 5.0.

**Results and Discussion**

**CD68**

In this study, the results of immunohistochemical analysis for CD68 have shown in table 2, 3 and 4. As shown in table 2 there significant correlation between CD68 with thyroid patients age less than (45 years) (P=0.01) and (P=0.0001) with thyroid patients age more than 45 years.
In table 3 and 4 the results recorded depended 2 scores weak positive and strong positive. There is significant difference between score of CD68 and gender (P.value: 0.058). (Table 3)

Score of CD68 showed associated with gender in table 2, from total 29 female were (20 cases, 98.9%) weak positive and (9 cases, 31%) strong positive while from 9 male were (5 cases, 55.6%) weak and (4 cases, 44.4%) strong positive.

Table 2: Distribution of thyroid carcinoma patients in relation with CD68 protein in immunohistochemical (IHC) method according to their age, gender and tumor type

<table>
<thead>
<tr>
<th>factor</th>
<th>CD68 +</th>
<th>CD68 -</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age total N</td>
<td>N %</td>
<td>N %</td>
</tr>
<tr>
<td>&lt;45 26</td>
<td>21 80.8</td>
<td>5 19.2</td>
</tr>
<tr>
<td>≥45 17</td>
<td>17 100</td>
<td>0 0</td>
</tr>
<tr>
<td>Sex N</td>
<td>N %</td>
<td>N %</td>
</tr>
<tr>
<td>Female 33</td>
<td>29 87.8</td>
<td>4 12.2</td>
</tr>
<tr>
<td>Male 10</td>
<td>9 90</td>
<td>1 10</td>
</tr>
<tr>
<td>Tumor type N</td>
<td>N %</td>
<td>N %</td>
</tr>
<tr>
<td>Benign 10</td>
<td>7 70</td>
<td>3 30</td>
</tr>
<tr>
<td>Malignant 33</td>
<td>31 93.9</td>
<td>2 6.1</td>
</tr>
<tr>
<td>Histotype 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>Papillary Ca 22</td>
<td>21 63.6</td>
<td>1 3.03</td>
</tr>
<tr>
<td>Follicular Ca 4</td>
<td>3 9.1</td>
<td>1 3.03</td>
</tr>
<tr>
<td>Medullary Ca 4</td>
<td>4 12.12</td>
<td>0 0</td>
</tr>
<tr>
<td>Anaplastic Ca 2</td>
<td>2 6.1</td>
<td>0 0</td>
</tr>
<tr>
<td>Hurthle cell 1</td>
<td>1 3.03</td>
<td>0 0</td>
</tr>
</tbody>
</table>

Table 4 showed CD68 score related with tumor type, PTC was appeared high associated with strong positive (11 cases, 28.9%) followed by MTC and Hurthle cell carcinoma in (1 case, 2.6) in same score. While the weak score was found in (7 cases, 18.5%) in benign tumors , (10 cases, 26.4%) in PTC , (3 cases, 7.9%) in FTC and MTC and finally (1 case ,4.5)in ATC.

Table 3: Immunohistochemical score of CD68 related with gender

<table>
<thead>
<tr>
<th>factor</th>
<th>CD68 IHC score</th>
<th></th>
<th>P.Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 25 weak</td>
<td>≥25 strong</td>
<td>0.058</td>
</tr>
<tr>
<td>Sex (+ve n)</td>
<td>No. %</td>
<td>No. %</td>
<td>Total</td>
</tr>
<tr>
<td>Female 29</td>
<td>20 69</td>
<td>9 31</td>
<td>100</td>
</tr>
<tr>
<td>Male 9</td>
<td>5 56</td>
<td>4 44</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>125</td>
<td>75</td>
<td>200</td>
</tr>
</tbody>
</table>
Table 4: Immunohistochemical score of CD68 related with tumor type

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>CD68 IHC score</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;25 weak</td>
<td>≥25 strong</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No. %</td>
<td>No. %</td>
<td></td>
</tr>
<tr>
<td>Benign</td>
<td>7 18.5</td>
<td>0 0</td>
<td></td>
</tr>
<tr>
<td>Malignat</td>
<td>No. %</td>
<td>No. %</td>
<td></td>
</tr>
<tr>
<td>Papillary Ca 21</td>
<td>10 26.4</td>
<td>11 28.9</td>
<td></td>
</tr>
<tr>
<td>Follicular Ca 3</td>
<td>3 7.9</td>
<td>0 0</td>
<td></td>
</tr>
<tr>
<td>Medullary Ca 4</td>
<td>3 7.9</td>
<td>1 2.6</td>
<td></td>
</tr>
<tr>
<td>Anaplastic Ca 2</td>
<td>2 5.4</td>
<td>0 0</td>
<td></td>
</tr>
<tr>
<td>Hurthle cell 1</td>
<td>0 0</td>
<td>1 2.6</td>
<td></td>
</tr>
</tbody>
</table>

P53

Expression of P53 was related with age group, gender and tumor type summarized in table 5. Of 26 patients were less than 45 year, only (5 cases, 11.6%) were positive. These 5 cases were 4 female and 1 male which of them 1 benign tumor case, (2, 6.1%) PTC, (1, 3.03%) FTC and (1, 3.03%) MTC.

Table 6 showed significant difference between score of P53 and gender (p.value:0.001).

The results in table 7 show that high percentage of strong score was in PTC (2 cases, 6.1%), followed by 1 case 3.03% strong score in FTC, 1 case 3.03% moderate score in benign and 1 case 3.03% in MTC.

Table 5: Distribution of thyroid carcinoma patients in relation with P53 protein in immunohistochemical (IHC) method according to their age, gender and tumor type

<table>
<thead>
<tr>
<th>factor</th>
<th>P53+</th>
<th>CDP53 -</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age total N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26 &lt;45</td>
<td>5 19.2</td>
<td>21 80.8</td>
</tr>
<tr>
<td>17 ≥45</td>
<td>0 0</td>
<td>17 100</td>
</tr>
<tr>
<td>Sex N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female 33</td>
<td>4 12.1</td>
<td>29 87.9</td>
</tr>
<tr>
<td>Male 10</td>
<td>1 10</td>
<td>9 90</td>
</tr>
<tr>
<td>tumor type N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>benign 10</td>
<td>1 10</td>
<td>9 90</td>
</tr>
<tr>
<td>malignant 33</td>
<td>4 12.1</td>
<td>29 87.9</td>
</tr>
<tr>
<td>histotype 0</td>
<td>0 0</td>
<td>0 0</td>
</tr>
<tr>
<td>Papillary Ca 22</td>
<td>2 6.1</td>
<td>20 60.6</td>
</tr>
<tr>
<td>follicular Ca 4</td>
<td>1 3.03</td>
<td>3 9.1</td>
</tr>
<tr>
<td>medullary Ca 4</td>
<td>1 3.03</td>
<td>3 9.1</td>
</tr>
<tr>
<td>Anaplastic Ca 2</td>
<td>0 0</td>
<td>2 6.1</td>
</tr>
<tr>
<td>Hurthle cell 1</td>
<td>0 0</td>
<td>1 3.01</td>
</tr>
</tbody>
</table>
Table 6: Immunohistochemical score of P53 related with gender

<table>
<thead>
<tr>
<th>Sex</th>
<th>P53 IHC score</th>
<th>P.value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>score 0 (-ve)</td>
<td>2 weak</td>
</tr>
<tr>
<td>Female</td>
<td>No. %</td>
<td>No. %</td>
</tr>
<tr>
<td>33</td>
<td>28</td>
<td>84</td>
</tr>
<tr>
<td>Male</td>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>Total</td>
<td>174</td>
<td>13</td>
</tr>
</tbody>
</table>

Discussion

The host’s immune response to the tumor represent by macrophage infiltrates in the context of or surrounding a variety of malignancies \(^4,7\). The “tumor-associated macrophages” (TAMs) had fundamental jobs in tumor development and metastasis of various cancers, including advanced thyroid malignant growth \(^17\). This study demonstrated the presence of CD68+ in benign and malignant thyroid tumors. But all (7 cases, 18.5%) of CD68+ in benign were in weak score. While (11 out of 21)28.9% from PTC were strong positive score which came in similar with Qing et al. \(^,2012\) who investigated TAM density in both benign thyroid tumors and PTC by CD68 immunostaining. They establish that overall density of TAM was higher compared with “thyroid goiter” and “follicular adenoma”. On the contrary to previous studies, \(^17\) pointed to the expression of TAM in 36 PTC Patient with L.N metastasis using immunohistochemical staining with anti-CD68 antibody. And Ryder et al. \(^,2008\) who recorded a higher TAM thickness in ineffectively separated PTC and ATC This different was explained by the mixed cell populates of macrophage with CD136 ,CD68 and undifferentiated monocyte/macrophage which are for all positive for CD68 ,these kinds of macrophages play special roles in the advancement and progression HNSC \(^18\). Other study by \(^10\) using CD68 immunohistochemistry, observed that TAM anticipated metastasis in a number of patients. Of 121 patients with PTC, 15% had TAMs (CD68) that seemed to have a phagocytic capacity on cancerous cells. These patients had fundamentally less blood vessel attack and remote metastasis and largely supplementary invasion lymphocyte and dendritic cells, than patients without a CD68 cells. Further, the findings of the current study are in similar with other study by \(^21\).

Who observed expression of p53 was more frequently in malignant than in benign lesions. Since p53 has been known to be rarely mutated in well differentiated tumors of the thyroid where an frequently expressed in poorly differentiated or undifferentiated tumors which are of different number, in our study, however there is a concerned of tumor suppressor gene where it plays an important role in cancer progression. Yet a serious studies should that it plays a role in early stages different types of cancers including thyroid cancers. We found a moderate immunoreactivity of Bcl2 in cytoplasm of epithelium of the benign and well-differentiated PTC and these findings are accordance with other studies by \(^21\).

Conclusion:

CD68 + macrophages are key players in thyroid tumors microenviroment leading to thyroid tumor development where its indirectly correlated with BCL2 family oncoproteins where antiapoptotic pathway is down regulated leading to increased aggregates of CD68+macrophages and that CD68+macrophages are positively correlated with p53 gene mutation expression where both markers can explain the biological behavior of tumor. BCL2&P53 have an inversed correlation since both have an opposite functions (p53 represent cell death gene while bcl2 is an antidote to a programmed cell death ,bcl2 was more expressed in benign tumor in our study than in malignant tumors unlike to p53.
where its more expressed in WDT (PTC 6% followed by FTC 3.03%) indicating that expression of p53 may be a late event in thyroid tumor genesis and that there’s a mutual expression between both bcl2 & p53 in thyroid tumors.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Dentistry, Mustansiriyah University, Iraq, Baghdad, Iraq and all experiments were carried out in accordance with approved guidelines.

References

19. Lu P, Lu Q, Rughetti A, et al. death and promotes the morphogenesis, but not tumorigenesis of


Prevalence of Lung Cancer in Non Smoker Patients Attending Al-Diwaniyah Teaching Hospital

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2 M.B.Ch.B/ Al-Diwaniyah Teaching Hospital / Al-Diwaniay Province/ Iraq

Abstract

Background: Smoking is a well known risk factor that is implicated in a number of human malignant disorders.

Aim of the study: To study the prevalence of lung cancer in a sample of Iraqi non smoker patients and the main risk factor implicated in such malignant tumor.

Patients and Method: In this hospital based study, in order to explore the prevalence rate of bronchogenic carcinoma in non smoker patients, it retrospectively collected data about those patients for the last four years, starting from January 2016 through June 2019. These information were retrieved from patients records that are already present in oncology and respiratory units in Al-Diwaniyah teaching hospital, Al-Diwaniyah province, Mid-Euphrates region of Iraq. The following variables were included in the questionnaire form: Age, gender, occupation, education level, residency, socioeconomic status, marital status history of smoking, history of second hand smoking, number of household smokers, type of smoking, history of alcoholism, history of chronic illness, the main presenting clinical features and clinical features of carcinoma and finding in examination as well as those obtained from investigations.

Results: In the current study, the prevalence rate of all cases of lung cancer during the period of study was 61 out of 403 (15.1 %). the rate of lung cancer in none smokers, in the current study, was 39.3 %. Old age individuals were the main age group affected and there was female predilection. there was also no significant difference in the distribution of patients and control subjects according to residency, occupation, level of education marital status and socioeconomic status (P > 0.05). In the current study, the prevalence rate of passive smoking in patients was significantly higher than that in control subjects, 70.8 % versus 40 %. Moreover, the number of household smokers was significantly higher inpatients group than in control group.

Conclusion: Lung cancer in non smokers appears to be significantly correlated with passive smoking in addition to increase incidence in old age and women.

Key words: Prevalence; Lung Cancer, Non Smoker, Iraq.

Introduction

The term, never smokers, refers to persons who have smoked fewer than 100 cigarettes in their lifetime, including lifetime nonsmokers. Most studies that track the trend of lung cancer rates often include both smokers and never smokers, and few studies independently study the trends over time for never smokers because of the limited longitudinal collection and the limited reliability of smoking information in population-based registries. From what is available, however, the overall global statistics estimate that 15% of lung cancers in men and up to 53% in women are not attributable to smoking, with never smokers accounting for 25% of all lung cancer cases worldwide 1. If lung cancer in never smokers were considered separately, it would rank as the seventh most common cause of cancer death worldwide before cervical, pancreatic, and prostate cancer 2. In countries in South Asia, up to 80% of women with lung cancer are never smokers 3. In the United States, one study estimated that 19% of lung cancer in women and 9% of
Lung cancer in men occurs in never smokers\(^4\). The age-adjusted rate for lung cancer in never smokers (ages 40–79 years) ranged from 11.2 to 13.7 per 100,000 person-years for men and from 15.2 to 20.8 per 100,000 person-years for women. The rates are 12 to 30 times higher in current smokers of the same age group. Lung cancer included heterogeneous group of malignant epithelial disorders that comprises squamous cell carcinoma, adenocarcinoma, small cell carcinoma and large cell carcinoma as well as a number of rare histopathological subtypes such as bronchoalveolar carcinoma. The most common subtype by far is squamous carcinoma and is mainly attributed to smoking but a number of cases have been reported in life time non smokers. Because of the shortage of Iraqi literatures dealing with lung cancer in non smokers since most published Iraqi articles deal with smoking related lung cancer, and because we are interested in making an idea about the prevalence rate of lung cancer in non smokers and to outline its possible risk factors in our community we planned and conducted the current study to one of leading articles in the Mid-Euphrates region dealing with this important human cancer.

**Patients and Method**

In this hospital based study, in order to explore the prevalence rate of bronchogenic carcinoma in non smoker patients we prospectively interviewed 5 cases during 2019 and retrospectively collected data about those patients for the last 3 years, starting from January 2016 through June 2018. These information were retrieved from patients records that are already present in oncology and respiratory units in Al-Diwaniyah teaching hospital, Al-Diwaniyah province, Mid-Euphrates region of Iraq. However for purpose of comparison in order to evaluate possible risk factors in association with non smoker bronchogenic carcinoma we included in the study 25 apparently healthy individuals serving as control group. Records of all patients with established diagnosis of bronchogenic carcinoma were included in the present study. Variables were registered concerning those patients who are never smokers. Although records of smoker patients with bronchogenic carcinoma were retrieved, information about them were used for calculation of prevalence rate of lung cancer in non smoker only, and the rest of information were excluded. The total number of patients with all kinds of malignant tumor was also obtained to calculate the prevalence rate of bronchogenic carcinoma out of all malignant tumors. The following variables were included in the questionnaire form: Age, gender, occupation, education level, residency, socioeconomic status, marital status, history of smoking, history of second hand smoking, number of household smokers, type of smoking, history of alcoholism, history of chronic illness, the main presenting clinical features and clinical features of carcinoma and finding in examination as well as those obtained from investigations. The study was approved by the institutional ethical approval committee and formal agreement was obtained from the directorate of Health in Al-Diwaniyah province, the formal representative of Iraqi Ministry of health. Data were collected and transformed into a spread sheet of Microsoft Office Excel 2010 and then into an SPSS (statistical package for social sciences) version 23. Numeric quantitative data were expressed as mean, range and standard deviation (SD), whereas, qualitative data were expressed as number and percentage. Comparison of mean between any two groups was done according to independent sample t-test, while chi-square test was used to evaluate association between any two categorical variables. The level of significance was considered at \( P \leq 0.05 \).

**Results**

During that period the number of cases with malignant tumors, including lung cancer, accounted for 403. In addition, the total number of cases with lung carcinoma during the period of study, irrespective of smoking, was 61. Therefore, the prevalence rate of all cases of lung cancer during the period of study was 61 out of 403 (15.1 %), out of which 24 cases were associated with evidence of passive smoking (39.3 %). Table 1 outlines the comparison of age between control and patients group. There was no significant difference in mean age between patient and control group, 61.63 ±15.31 years versus 68.28 ±8.50 years, respectively (\( P = 0.065 \)). The age range of patients with bronchogenic carcinoma was form 30 – 85 years. In addition the frequency distribution of patients according to 10 years intervals was as following: 8.3 %, 16.7 %, 4.2 %, 41.7 %, 12.5 % and 16.7 % as 30-39 years, 40-49 years, 50-59 years, 60-69 years, 70-79 years and ≥ 80 years, respectively. Thus, the highest incidence rate was observed at age interval of 60-69 years, table 1.
Table 1: Mean age and age range of patients and control subjects

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Control group n = 25</th>
<th>Patients group n = 24</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-39</td>
<td>0 (0.0)</td>
<td>2 (8.3)</td>
<td></td>
</tr>
<tr>
<td>40-49</td>
<td>0 (0.0)</td>
<td>4 (16.7)</td>
<td></td>
</tr>
<tr>
<td>50-59</td>
<td>2 (8.0)</td>
<td>1 (4.2)</td>
<td></td>
</tr>
<tr>
<td>60-69</td>
<td>13 (52.0)</td>
<td>10 (41.7)</td>
<td></td>
</tr>
<tr>
<td>70-79</td>
<td>6 (24.0)</td>
<td>3 (12.5)</td>
<td></td>
</tr>
<tr>
<td>≥ 80</td>
<td>4 (16.0)</td>
<td>4 (16.7)</td>
<td></td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>68.28 ±8.50</td>
<td>61.63 ±15.31</td>
<td>0.065†</td>
</tr>
<tr>
<td>Range</td>
<td>55 - 90</td>
<td>30 - 85</td>
<td>NS</td>
</tr>
</tbody>
</table>

n: number; data were presented as mean standard deviation or number (%); †: independent samples t-test; NS: not significant at P ≤ 0.05

With respect to gender, women accounted to 15 out 24 patients with bronchogenic carcinoma thereby accounting for 62.5 %, making the male to female ratio 1:1.7. Control group included 12 men and 13 women; therefore there was no significant difference in the distribution of patients and control subjects according to gender, as shown in table 2. There was also no significant difference in the distribution of patients and control subjects according to residency, occupation, level of education marital status and socioeconomic status (P > 0.05), as shown in table 3. Regarding bad habits, none of patients or control subjects admit to be alcoholic of experience cigarette smoking.

Table 2: Frequency distribution of patients and control subjects according to gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>Control group n = 25</th>
<th>Patients group n = 24</th>
<th>χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n %</td>
<td>n %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>12 48</td>
<td>9 37.5</td>
<td>0.551</td>
<td>0.458† NS</td>
</tr>
<tr>
<td>Female</td>
<td>13 52</td>
<td>15 62.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n: number of cases; †: Chi-square test; NS: not significant at P ≤ 0.05

Table 3: Demographic characteristics of control subjects and patients with bronchogenic carcinoma

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control group n = 25</th>
<th>Patients group n = 24</th>
<th>χ²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% n</td>
<td>% n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residency</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>19 76.0</td>
<td>14 58.3</td>
<td>1.738</td>
<td>0.187† NS</td>
</tr>
<tr>
<td>Rural</td>
<td>6 24.0</td>
<td>10 41.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employee</td>
<td>4 16.0</td>
<td>3 12.5</td>
<td>5.654</td>
<td>0.341† NV</td>
</tr>
<tr>
<td>Farmer</td>
<td>0 0.0</td>
<td>4 16.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Housewife</td>
<td>10 40.0</td>
<td>7 29.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Private Job</td>
<td>1 4.0</td>
<td>2 8.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Teacher</td>
<td>4 16.0</td>
<td>2 8.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No job</td>
<td>6 24.0</td>
<td>6 25.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 3: Demographic characteristics of control subjects and patients with bronchogenic carcinoma

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control group</th>
<th>Patients group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 25</td>
<td>n = 24</td>
</tr>
<tr>
<td>Illiterate</td>
<td>13</td>
<td>52.0</td>
</tr>
<tr>
<td>Primary</td>
<td>2</td>
<td>8.0</td>
</tr>
<tr>
<td>Secondary</td>
<td>3</td>
<td>12.0</td>
</tr>
<tr>
<td>University</td>
<td>7</td>
<td>28.0</td>
</tr>
<tr>
<td>Single</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Married</td>
<td>25</td>
<td>100.0</td>
</tr>
<tr>
<td>Poor</td>
<td>2</td>
<td>8.0</td>
</tr>
<tr>
<td>Moderate</td>
<td>11</td>
<td>44.0</td>
</tr>
<tr>
<td>Good</td>
<td>12</td>
<td>48.0</td>
</tr>
</tbody>
</table>

n: number of cases; ¥: Chi-square test; F: Fischer exact test; NS: not significant; NV: not valid since > 20% of cells have expected count < 5.

### Table 4: Chronic disorders in control and patients with bronchogenic carcinoma

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control group</th>
<th>Patients group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 25</td>
<td>n = 24</td>
</tr>
<tr>
<td>HT</td>
<td>14</td>
<td>56.0</td>
</tr>
<tr>
<td>DM</td>
<td>8</td>
<td>32.0</td>
</tr>
<tr>
<td>IHD</td>
<td>1</td>
<td>4.0</td>
</tr>
<tr>
<td>Asthma</td>
<td>3</td>
<td>12.0</td>
</tr>
</tbody>
</table>

n: number of cases; ¥: Chi-square test; F: Fischer exact test; NS: not significant; S: significant at \( P \leq 0.05 \)

### Table 5: Prevalence rate of passive smoking in patients and control subjects

<table>
<thead>
<tr>
<th>Passive smoking</th>
<th>Control group</th>
<th>Patients group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 25</td>
<td>n = 24</td>
</tr>
<tr>
<td>Positive</td>
<td>10</td>
<td>40.0</td>
</tr>
<tr>
<td>Negative</td>
<td>15</td>
<td>60.0</td>
</tr>
</tbody>
</table>

n: number of cases; ¥: Chi-square test; S: significant at \( P \leq 0.05 \)
Table 6: Number of household smokers in patients and control groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SD (IQR)</th>
<th>Median (IQR)</th>
<th>Range</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>0.64 ±0.86 (2.00)</td>
<td>0.00 - 2.00</td>
<td>0.00 - 2.00</td>
<td>0.027†</td>
</tr>
<tr>
<td>Patients group</td>
<td>1.38 ±1.25 (3.00)</td>
<td>1.00 - 4.00</td>
<td>0.00 - 4.00</td>
<td>S</td>
</tr>
</tbody>
</table>

SD: standard deviation; IQR: inter-quartile range; †: Mann Whitney U test; S: significant at P ≤ 0.05

Discussion

In the current study, the prevalence rate of all cases of lung cancer during the period of study was 61 out of 403 (15.1 %). It has been estimated that lung cancer accounts for about 16 % of all cancers, a proportion that is nearly similar and in total agreement with our finding. On the other hand, the rate of lung cancer in none smokers, in the current study, was 39.3 %. Most published articles indicated that the proportion of non smokers with lung cancer ranges between 10 to 25 % (6,7, 8,9). This indicates that a significant proportion of patients with bronchogenic carcinoma, in our study, have acquired the disease irrespective with cigarette smoking. Therefore, identifying other possible risk factors in association with lung cancer in those patients was the main aim of the current study. Indeed, we supposed that passive smoking may play a principal role in the development of lung cancer in patients who are not habitual smokers. Unfortunately lung cancer in never-smokers has been studied far less extensively than tobacco-related lung cancer. This indicates that age by itself may be a risk factor. Indeed, most published articles dealing with lung cancer in non smokers has linked age as a potential risk factor for such malignant disorder (10, 11,12,13). Older age is associated with cancer development due to biologic factors that include DNA damage over time and shortening telomeres. Accordingly, the median age of lung cancer diagnosis is 70 years for both men and women. On the other hand and with respect to gender, in the current study, women accounted to 15 out 24 patients with bronchogenic carcinoma thereby accounting for 62.5 %, making the male to female ratio 1:1.7. There are conflicting data regarding the possibility that women may be more susceptible to developing lung cancer 15. There is a higher rate of lung cancer in non-smoking women compared with non-smoking men, a higher proportion of epidermal growth factor receptor (EGFR) mutations in female NSCLC, and a higher incidence of adenocarcinoma with lepidic features in women (16,17). Some genetic mutations found to be more common in females may predispose toward lung cancer development in women, including over-expression of the CYP1A1 gene, mutation of the glutathione S-transferase M1 enzyme, mutations of the p53 tumor suppressor gene, and over-expression of X-linked gastrin-releasing peptide receptor (15, 16,17). Moreover, in the current study, there was also no significant difference in the distribution of patients and control subjects according to residency, occupation, level of education marital status and socioeconomic status (P > 0.05). In agreement with our results, a number of authors have denied any association between residency, rural versus urban and lung cancer (18,19). Exposure to pollutant in association with occupation is the main hazard precipitating to lung cancer. The occupational and environmental exposure to carcinogenic agents is an everyday phenomenon.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Medicine/ University of Al-Qadisiyah, Iraq and all experiments were carried out in accordance with approved guidelines.

References


Aging Influence Mitochondrial Dysfunction and Oxidative Stress through Check Some Proinflammatory Cytokines Levels and Oxidant Status in Older Adults: an Observational Study

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1Sulaimany Technical Institute, Sulaimany Polytechnic University, Sulaimany, Iraq, 2Biology Department, Faculty of Science and Health, Department of biology, Faculty of science & Health, Koya University, Koya KOY45, Kurdistan Region – F.R. Iraq

Abstract

The experiment design is that of a cohort study within the setting of a medium private laboratory with participants being common people (healthy individuals of 20–99 years=108; mean age 55 years and 71.3% man) were classified into six age groups. Oxygen saturation (SpO2), pulse rate, systolic blood pressure (CSBP) and diastolic blood pressure were assessed. Blood sera (once) was obtained for proinflammatory cytokines levels (including c-reactive protein (CRP), insulin growth factor-1 (IGF-1), apolipoprotein E (apoE), interleukin 6 (IL-6), 8-hydroxy-2’–deoxyguanosine (8-OHdG) and tumor necrosis factor-α (TNF-α)) and oxidant status (including malondialdehyde (MDA), superoxide dismutase (SOD) and total antioxidant capacity (T-AOC) analysis. The mean calculated of the whole participants were equal for proinflammatory cytokines levels: CRP 3.24 mg/dL, IGF-1 54.03 ng/mL, apoE 488.29 mg/mL, IL-6 65.464 pg/mL, 8-OHdG 1.34 ng/mL and TNF-α 105.63 pg/mL; and oxidant statue: MDA 0.59 ng/mL, SOD 3.48 U/mL and T-AOC 1.21 mmol/L, receptively. Our findings highlighted that survey of mitochondrial dysfunction and oxidative stress through check some proinflammatory cytokines levels and oxidant status of aging in older adults can be applied to build a global frailty index as a tool to quantify aging in preclinical experiments.

Keywords: Physiological aging, Proinflammatory cytokines, Oxidant status, Adults, Iraq

Introduction

The world population continues to grow older rapidly, mostly because of declining fertility and increasing longevity. For this reason, aging research has experienced an unprecedented advance over recent years, particularly with the discovery that the rate of aging is controlled, at least to some extent, by biochemical processes and genetic pathways conserved in evolution.

Aging is a process of the progressive functional decline of various physiological functions in various organs and tissues with time, which lead to disability, dependence, morbidity, and mortality. This reduction manifests as a decreased physiological reserve in response to a time-dependent failure of complex molecular mechanisms and stress (termed homeostenosis) that cumulatively create disorder.

Given the intricacy of the ageing process in organism, the underlying exact mechanisms are still not good understood. In current literature, free radical theory of aging is the most intriguing among the various mechanisms that are postulated to participate in the course of aging. And, mitochondria are important determinants of cellular homeostasis and longevity since they are the main producers of cellular ATP and play a vital role in regulation of apoptotic death pathways in many tissues. And, based on free radical theory of aging mitochondria are the origin source of intracellular reactive oxygen species (ROS, which are not readily

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Email: kaniaw.khafar@spu.edu.iq
removed) in normal human aging. ROS accumulate as side products of the electron transport chain, causing mitochondrial dysfunction.  

In the past decade, efforts to develop an aging metric based on genetic profiles, epigenetic changes and telomere length have met with limited success. Thus, inflammatory cytokines and oxidant status proportion in the blood may be practical to monitoring those at risk of cognitive and functional decline in older adults aging. The main aim this experiment a set of unifying hallmarks of free radical theory of aging that has been defined providing an outline for understanding the process of mitochondrial ageing and oxidative at older adults function through check some proinflammatory cytokines levels and oxidant status.

**Materials and Method**

**Experimental design**

The experiment design is that of a cohort study within the setting of a medium private laboratory with participants being common people (healthy individuals of 20–99 years=108; mean age 55 years and 71.3% man) were classified into six groups according to age of participants (years): I) 20-39 (n=15); II) 40-49 (n=24); III) 50-59 (n=23); IV) 60-69 (n=22); V) 70-79 (n=13) and VI) 80-99 (n=11). All samples were obtained from Sulaimani Nursing House and New Medical Center (Private Laboratory) from December-2016 to April-2017. Experimental protocols were approved by the Ethics Review Committee of Medicine, Sulaimani Nursing House, Iraq (approval no: 0529.1.75/3).

**Data collection**

Reagents and equipment were purchased from Roche (Germany). Before blood sampling, operators medical were took some clinical examination from participants including oxygen saturation variability (as described previously by), pulse rate, systolic blood pressure (SBP) and diastolic blood pressure (DBP) by automated Watch Allyn sphygmomanometer (Germany). Fasting blood samples (once) were obtained of all participants into vacuum tubes (non-heparinized) for the assay of serum. The serum was separated by centrifugation (15,000×g for 10 min), kept at −80 °C and assessed by Human ELISAs kit, as recommended by the manufacturer.

**Sera proinflammatory cytokines and oxidant measurements**

The sample characteristics of proinflammatory cytokines and oxidant statue kits are shown in Table 1. Blood sera was obtained for assay proinflammatory cytokines levels (including c-reactive protein (CRP), insulin growth factor-1 (IGF-1), apolipoprotein E (apoE), IL-6, 8-hydroxy-2′–deoxyguanosine (8-OHdG), and tumor necrosis factor-α TNF-α) and oxidant statue (including MDA, SOD, and total antioxidant capacity (T-AOC)) analysis.

**Table 1: Sample characteristics of proinflammatory cytokines and antioxidant kits**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cat number</th>
<th>Sensitivity</th>
<th>Range</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (mg/L)</td>
<td>cat# 20764930322</td>
<td>&lt;5.0</td>
<td>1.0-200</td>
<td>Roche Cobas Integra 400 plus</td>
</tr>
<tr>
<td>IGF-1 (ng/mL)</td>
<td>cat# EELH0086</td>
<td>&lt;0.94</td>
<td>1.56–100</td>
<td>Elabscience Biotechnology Inc.</td>
</tr>
<tr>
<td>apoE (ng/mL)</td>
<td>cat# EELH0470</td>
<td>&lt;14.06</td>
<td>23.44-1500</td>
<td>Elabscience Biotechnology Inc.</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>cat# EELH0102</td>
<td>&lt;4.69</td>
<td>7.81-500</td>
<td>Elabscience Biotechnology Inc.</td>
</tr>
<tr>
<td>8-OHdG (ng/mL)</td>
<td>cat# EEL0028</td>
<td>&lt;0.938</td>
<td>1.563-100</td>
<td>Elabscience Biotechnology Inc.</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>cat# EELH0109</td>
<td>&lt;4.69</td>
<td>7.81-500</td>
<td>Elabscience Biotechnology Inc.</td>
</tr>
<tr>
<td>MDA (ng/mL)</td>
<td>cat# EELH0060</td>
<td>&lt;18.75</td>
<td>31.25–2000</td>
<td>Elabscience Biotechnology Inc.</td>
</tr>
<tr>
<td>SOD (U/mL)</td>
<td>cat# BC0020</td>
<td>0.2</td>
<td>0.2-14.4</td>
<td>Elabscience Biotechnology Inc.</td>
</tr>
<tr>
<td>T-AOC (mmol/L)</td>
<td>cat# BC0219</td>
<td>0.047</td>
<td>0.047-1.5</td>
<td>Elabscience Biotechnology Inc.</td>
</tr>
</tbody>
</table>

CRP: c-reactive protein; IGF-1: insulin growth factor-1; apoE: apolipoprotein E; IL-6: interleukin 6; 8-OHdG: 8-hydroxy-2′–deoxyguanosine; TNF-α: tumor necrosis factor-α; MDA: malondialdehyde; SOD: super oxidase dismutase; T-AOC: total antioxidant capacity
Statistical Analysis

The results were analyzed using the SPSS 22 software. ANOVA and post-hoc LSD test was used to test differences between treatments. The results were expressed as the mean ± standard deviation (SD). Differences with values of \( p<0.05 \) were considered statistically significant.

Results

No participant recruited chose to withdraw from the experiment then all participants were considered for analysis. The descriptive statistics presented in Table 1, show that the highest (97.4±0.99) and least (95.45±2.77) mean of oxygen saturation (SpO2) in groups of 20-39 and 80-99 years, respectively. And, the only significant differences were detected between the groups 70-79 and 80-99 years (\( p<0.05 \)). The analysis revealed that significant differences for pulse rate (\( p=0.037 \)) and systolic blood pressure (\( p=0.005 \)) were detected between the means of the age groups, but the pattern was not consistent. The mean pulse rate of the whole participants was 78.06 beat/minute. Higher means were detected in the young and the old age groups, and the lowest were observed in the 50-69 years group. The highest (136.82±11.89) and least (119.71±13.2) mean of systolic blood pressure (mmHg) were shown in groups of 80-99 and 40-49 years, respectively. The diastolic blood pressure of the whole sample was 84 mmHg. The highest mean (107.83±12.5) diastolic blood pressure (mmHg) was in the age group 50-59 years, but the difference was not significant (\( p=0.482 \)).

Table 2: Mean ±SD of SpO2, pulse rate, SBP and DBP, stratified by age

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>SpO2 (%)</td>
<td>97.40±0.99a</td>
<td>97.29±0.91a</td>
<td>97.00±0.74a</td>
<td>97.18±1.22a</td>
<td>96.00±1.78b</td>
<td>95.45±2.77b</td>
</tr>
<tr>
<td>Pulse rate (%)</td>
<td>83.47±10.41a</td>
<td>80.08±14.68ab</td>
<td>74.78±9.86 ab</td>
<td>72.41±10.62b</td>
<td>81.31±13.58 ab</td>
<td>80.55±8.69 ab</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>126.7±11.75ab</td>
<td>119.7±13.20a</td>
<td>135.7±18.61b</td>
<td>134.8±15.47b</td>
<td>132.5±21.55b</td>
<td>136.8±11.89b</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>77.33±8.84</td>
<td>76.54±7.38</td>
<td>81.52±11.02</td>
<td>77.95±10.87</td>
<td>77.69±10.53</td>
<td>79.09±7.35</td>
</tr>
</tbody>
</table>

SpO2: oxygen saturation; SBP: systolic blood pressure; DBP: diastolic blood pressure

The same letters mean non-significant difference, while the different letters mean significant difference at \( p<0.05 \).

Table 3 and 4 provides a summary of the outcomes for sera levels of proinflammatory cytokines and oxidant statue by treatment group and study time points. The mean calculated of the whole participants were equal for proinflammatory cytokines levels: CRP 3.24 mg/L, IGF-1 54.03 ng/mL, apoE 488.29 mg/mL, IL-6 65.46 pg/mL, 8-OHdG 1.34 ng/mL and TNF-α 105.63 pg/mL; and oxidant statue: MDA 0.59 ng/mL, SOD 3.48 U/mL and T-AOC 1.21 mmol/L, receptively. Several parameters significantly differed between age groups. The blood sera analysis in all age groups revealed that the levels of some proinflammatory cytokines (except IGF-1) and oxidant statue (except MDA) adults in middle to late adulthood significantly decreased \( (p<0.001) \). The kits analysis revealed that the levels of IGF-1 (chemiluminescent/immunometric assay) and MDA (colorimetric/fluorometric assay) adults in middle to late adulthood decreased and increased, receptively \( (p<0.001) \). LSD test showed that mean of IGF-1 the significant difference between each of two groups except for the difference between groups of 70-79 and 80-99 years \( (p=0.244) \). In contrary, mean of MDA the significant difference between each of two groups except for the difference between groups of 20-39 and 40-49 years \( (p=0.975) \).
Table 3: Serum levels of CRP, IGF-1, APO-E, IL-6, 8-OHdG and TNF-α, stratified by age

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (mg/dL)</td>
<td>2.44±0.98a</td>
<td>2.82±1.84ab</td>
<td>3.14±1.60 ab</td>
<td>3.11±1.25ab</td>
<td>3.74±1.42b</td>
<td>5.06±1.41c</td>
</tr>
<tr>
<td>IGF-1 (ng/mL)</td>
<td>78.36±7.14a</td>
<td>71.35±6.44b</td>
<td>60.06±4.75c</td>
<td>51.56±2.97d</td>
<td>40.23±4.37e</td>
<td>38.15±7.70c</td>
</tr>
<tr>
<td>APO-E (mg/mL)</td>
<td>251.9±74.8a</td>
<td>383.0±66.84b</td>
<td>409.59±80.18b</td>
<td>573.4±102.6c</td>
<td>668.1±87.70d</td>
<td>821.9±77.68e</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>46.73±4.07a</td>
<td>51.24±3.87b</td>
<td>60.69±6.62c</td>
<td>70.51±5.59d</td>
<td>87.90±6.32e</td>
<td>95.45±4.62f</td>
</tr>
<tr>
<td>8-OHdG (ng/mL)</td>
<td>0.43±0.08a</td>
<td>0.89±0.08b</td>
<td>1.03±0.07c</td>
<td>1.40±0.08d</td>
<td>1.88±0.08e</td>
<td>2.38±0.10f</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>59.92±7.44a</td>
<td>75.43±8.78b</td>
<td>91.33±8.66c</td>
<td>121.13±15.1d</td>
<td>157.5±8.85c</td>
<td>171.5±17.42f</td>
</tr>
</tbody>
</table>

CRP: c-reactive protein; IGF-1: insulin growth factor-1; apoE: apolipoprotein E; IL-6: interleukin 6; 8-OHdG: 8-hydroxy-2’–deoxyguanosine; TNF-α: tumor necrosis factor-α.

Note: Results expressed as Mean ±SD. The same letters mean non-significant difference, while the different letters mean significant difference at p<0.05

Table 4: Serum levels of MDA, SOD and T-AOC, stratified by age

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (ng/mL)</td>
<td>0.45±0.09a</td>
<td>0.45±0.06a</td>
<td>0.55±0.04b</td>
<td>0.64±0.06c</td>
<td>0.73±0.10d</td>
<td>0.91±0.07e</td>
</tr>
<tr>
<td>SOD (U/mL)</td>
<td>3.95±0.68 a</td>
<td>3.99±0.42 a</td>
<td>3.49±0.63 b</td>
<td>3.18±0.62 b</td>
<td>3.18±0.46 b</td>
<td>2.64±0.23c</td>
</tr>
<tr>
<td>T-AOC (mmol/L)</td>
<td>1.42±0.32a</td>
<td>1.41±0.14a</td>
<td>1.27±0.11b</td>
<td>1.16±0.10b</td>
<td>1.04±0.10c</td>
<td>0.67±0.13d</td>
</tr>
</tbody>
</table>

MDA: malondialdehyde; SOD: super oxidase dismutase; T-AOC: total antioxidant capacity

Note: Results expressed as Mean ±SD. The same letters mean non-significant difference, while the different letters mean significant difference at p<0.05

Discussion

Pulse oximetry is a method used to quantify SpO2 non-invasively. And, it is a technique regularly utilized clinically whether that be in concentrated consideration, in medical procedure, or in some out-patient clinics. The results current experiment showed no substantial variance between groups in association with SpO2, only a slight decreasing was found in group of 80-90 years age, and it’s in agreement with the study of in which the SpO2 showed no substantial variance in groups under 35 ages and above 50 ages. Other previous studies indicated that there were a number of ways in which free radicals are formed but their most abundant source are the mitochondria (which uses some 90% of the O2 used by the human body) where oxygen is reduced in sequential steps to produce water. CRP functions as a marker of systemic inflammation and is one of the top studied inflammatory biomarkers in coronary arterial disease. According to the current edition of Harrison’s Principles of Internal Medicine (2012) the normal range of CRP 0.2–3.0 mg/L, while the presently obtained results showed that adults in middle to late adulthood showed higher serum CRP levels 3.0-5.0 mg/L (Table 3). Importantly, the population differences should also be taken into multiple factors including genetics, environmental conditions, developmental programming determine maximal organ function, diet and polymorphism which varies significantly between individuals.
In summary, described that IGF-1 the cross road of the nutritional, inflammatory and hormonal pathways to frailty. And, targeted deletion of specific genes has demonstrated that multiple components of the IGF-1/insulin signaling pathway play a role in the aging process spanning from nematodes to rodents. This hormone endorses cell survival, prevents apoptosis, and motivates neurogenesis in the hippocampus that affected early in Alzheimer disease. The results of our investigation showed that the minimum IGF-1 (ng/mL) mean (38.15) was observed in group 80-99 years age, while the maximum mean (78.36) was displayed in group 20-39 years age (Table 3).

Previous studies indicated that apoE is a useful player in studies of longevity and age-related diseases, such as inflammatory status and atherosclerosis that are known risk factors for functional decline and early mortality. Moreover, it is possible that apoE may also play a role in other pathological conditions including, for example, cancer, rheumatoid arthritis and macular degeneration, but the exact biological mechanisms underlying these observations are poorly understood until now. Our study observed the least (251.97±74.48) and highest (821.98±77.68) mean of apoE (ng/mL) were shown in groups of 20-39 and 80-99 years, respectively (Table 3).

Measuring biomarker signatures of inflammation, such as IL-6, TNF-α, and IL-1β that change with aging may be an effective way to assess for inflammaging in older adults and thus risk for inflammatory diseases with high morbidity and mortality rates. The results observed in the present study that extreme mean of IL-6 (95.45 pg/mL) and TNF-α (171.46 pg/mL) in group 80-99 years, and the minimum mean IL-6 (46.73 pg/mL) and TNF-α (59.92 pg/mL) in group 20-39 years age (Table 3).

**Conclusion**

This study might provide a therapeutic target for aging and age-related disease. On the other hand, “check some proinflammatory cytokines levels and oxidant status”, can be itself considered a hallmark of ageing.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under Sulaimany Technical Institute, Sulaimany Polytechnic University, Sulaimany, Iraq and all experiments were carried out in accordance with approved guidelines.

**References**


27. Lee EG, Son H. Adult hippocampal neurogenesis and related neurotrophic factors. BMB Reports, 2009;42(5):239-244.
Robust Bayesian Estimators For Survival Function Under Prior Data Conflict With Practical Application in the Health Side

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Abstract

The analysis of survival functions is concerned with knowing how long humans will survive. This means studying and analyzing the time from the beginning of the disease to the end point of death. For example, the survival function of patients with heart attacks was studied and analyzed at Al Manathira General Hospital. The Weibull distribution was used to match the real data. The scale parameter & survival function have been estimated for Weibull distribution with have two parameters, this distribution was used in two cases, prior data unconflict & prior data conflict. A regular Bayes method & robust Bayes were used for estimation. We used inverse gamma distribution as a prior where it is a conjugate prior for Weibull distribution. Two simulation experiments have been used; the first experiment used was prior data unconflict where the regular Bayes method is the best for estimating the scale & survival function by using the integrated mean square error (IMSE) as a criterion for comparing. The second experiment is in the case of prior data conflict. The results showed that the robust Bayes method is the best for estimation of the scale parameter & survival function by using (IMSE).

Keyword: Robust Bayesian, Prior data conflict, Survival function, iLuck Model, Regular Bayesian

Introduction

The statistical inference in the Bayesian method also relies on the prior information or the so-called prior distribution so that the prior distribution is combined with the distribution of observations according to the base of the Bayes rule so we get the posterior distribution from here, a problem might appear, which is the prior data conflict (prior data are the default values For the prior distribution parameters), ⁶. In the sense that the prior data does not necessarily correspond with the views or sample under study, and because of that you should know the existence of this problem or not when using the methods of Bayesian estimation, & to know the existence of this problem by modeling the parameters of the prior distribution, ⁸. So that the prior distribution should be the conjugate prior, & then after modeling the prior distribution parameters we produce the standard deviation of the prior distribution & the standard deviation of the posterior distribution, ¹¹. If the value of the standard deviation of the prior distribution is greater than the standard deviation of the posterior distribution, then there is a problem, ¹⁰. Hence, the main objective of our research is to obtain the best estimate of the survival function under prior data conflict by addressing this problem by assuming a set of prior information to obtain a set of prior distributions & thus we will obtain a set of posterior distributions, ⁷.⁸ After that, we obtain the estimates that are more efficient & accurate so that the method is called the robust Bayesian estimation, where a two-parameter Weibull distribution will be used to estimate the parameter of scale parameter & survival function because it is considered to be the most common distributions of survival models. ⁹ So that the use of the usual Bayesian method & the robust Bayesian method for estimating the scale parameter & survival function & the IMSE will be used to compare these methods.

The estimation of methods

We will estimate the survival function for Weibull distribution & the scale parameter λ & consider the shape parameter β is known, in the Bayesian & robust Bayesian method as shown below:
\[ f(t) = \frac{\beta}{\lambda} t^{\beta-1} e^{-\frac{t^\beta}{\lambda}} \]  

(1)

The prior distribution of the parameter \( \lambda \) will be used, which is inverse gamma:

\[ f(\lambda | a, b) = \frac{b^a}{\Gamma(a)} \lambda^{-a-1} e^{-\frac{b}{\lambda}} \]  

(2)

Then we get the posterior distribution as follows:

\[ f(\lambda | t) = \frac{\left( \sum t_i^\beta + b \right)^{(a+n)}}{\Gamma(a+n)} \lambda^{-(a+n)-1} e^{-\frac{(\sum t_i^\beta + b)}{\lambda}} \]  

(3)

The above equation is the posterior distribution of parameter \( \lambda \), & according to the quadratic loss function mean that the posterior distribution represents the Bayes estimator of parameter \( \lambda \) as shown below:\footnote{3}.

\[ \hat{\lambda} = \frac{(b + \tau(t))}{a + n} \]  

(4)

where \( \tau(t) = \sum t^\beta \)

2-1-2: Bayesian Estimation for Survival function

The estimation of the survival function & it is based on the quadratic loss function as shown below by:\footnote{2}:

\[ \hat{S}(t) = \int_0^\infty S(t) h(\lambda | t) \, d\lambda \]

\[ \hat{S}(t) = \left( \frac{b + \tau(t)}{b + \tau(t) + t^\beta} \right)^{a+n} \]  

(5)

Robust Bayesian Method

Checking for prior data conflict

Suppose that we have a sample distributed with the Weibull distribution & concisely \( t \sim \text{wei}(\beta, \lambda) \) as shown below:\footnote{11,12}:

\[ f(t | \lambda, \beta) = \frac{\beta}{\lambda} t^{\beta-1} e^{-\frac{t^\beta}{\lambda}} \]

The prior distribution of the scale parameter \( \lambda \) is inverse gamma because it is conjugate prior as shown below:

\[ f(\lambda | a, b) = \frac{b^a}{\Gamma(a)} \lambda^{-a-1} e^{-\frac{b}{\lambda}} \]

Then the prior parameters need to update so that it is \((n^0 > 1, y^0 > 0)\) instead of the parameters \((a, b)\), through two methods, we get the prior distribution as shown below:

\[ E(\lambda | a, b) = y^0 = \frac{b}{a-1} = \frac{b}{n^0} \Rightarrow b = n^0 y^0, n^0 = a - 1 \Rightarrow a = n^0 + 1 \]
After that, the prior distribution is obtained by the parameters $n^0$, $y^0$, through testing the problem of prior-data conflict as shown below:

$$f(\lambda/n^0y^0) = \frac{(n^0y^0)^{n^0+1}}{r(n^0+1)} \lambda^{-(n^0+1)-1} e^{-\frac{n^0y^0}{\lambda}}$$  \hspace{1cm} (6)

$y^0$: The prior guessing for the scale parameter $\lambda$.

$n^0$: The prior guessing to sample size $n$.

The equation above represents the distribution of prior by parameters $n^0$, $y^0$, which is also inverse gamma. After we obtain the prior distribution with parameters $n^0$, $y^0$, we derive the standard deviation of this distribution.

$$M_r = \frac{(n^0y^0)^r}{r(n^0+1)} r(n^0 - r + 1)$$  \hspace{1cm} (7)

$$s.d\ prior = \sqrt{\frac{(y^0)^2}{1-n^0}}$$  \hspace{1cm} (8)

The equation above represents the standard deviation of the prior distribution, & then the posterior distribution is derived as shown below:

$$f(\lambda|t) = \frac{(n^0y^0+\tau(t))^{n^0+n+1}}{r(n^0+n+1)} \lambda^{-(n^0+n+1)-1} e^{-\frac{(n^0y^0+\tau(t))}{\lambda}}$$  \hspace{1cm} (9)

After the posterior distribution is obtained & according to the equation above we derive the standard deviation for the posterior distribution as shown below:

$$M_r = \frac{(n^0y^0+\tau(t))^r}{r(n^0+n+1)} r(n^0 + n - r + 1)$$  \hspace{1cm} (10)

$$s.d\ posterior = \sqrt{\frac{(n^0y^0+\tau(t))^2}{(n^0+n)(n^0+n-1)}}$$  \hspace{1cm} (11)

Equation (13) represents the standard deviation of the posterior distribution to compare the standard deviation of the prior distribution of parameters $n^0$, $y^0$ with the standard deviation of the posterior distribution. If the standard deviation of the prior distribution is greater than the standard deviation of the posterior distribution, this means a prior-data conflict. This problem is needed to solve through the following steps.
A second way to obtain variance for posterior distribution is through the steps below:

As \( f(\lambda/t, n^0, y^0) = f(\lambda/n^ny^n) \) It means:

\[
f(\lambda/t, n^0, y^0) = f(\lambda/n^ny^n) = \frac{(n^0y^n)n^{n+1}}{t(n^{n+1})} \lambda^{-(n^{n+1})-1} e^{-\frac{n^0y^n}{\lambda}}
\]  

(12)

The variance of this distribution is:

\[
V(\lambda/n^n, y^n) = \frac{(y^n)^2}{1 - \frac{1}{n^n}}
\]

Therefore, the standard deviation of the prior distribution & the standard deviation of the posterior distribution can be written according to the following formula:

\[
s.d \text{ prior} = \sqrt{\frac{(y^0)^2}{1 - \frac{1}{n^0}}}
\]  

(13)

\[
s.d \text{ posterior} = \sqrt{\frac{(y^n)^2}{1 - \frac{1}{n^n}}}
\]  

(14)

Address the problem of prior data conflict

Although this is a problem of prior-data conflict, a model can be presented to address the problem of prior-data conflict, through the submission of a set of parameters prior as suggested by 5. Through the following model \( \prod_0^n = n^0x[y^0, y^0] \). Another suggestion was made by 7. For result a set of prior parameters through the model \( \prod_0^n = [n^0, \bar{n}^0]x[y^0, y^0] \). In general the model submitted for resulting a set of prior parameters is called (generalized iLuck-Model). Then we get the updated parameters to be more accurate as shown in the following steps 10:

\[
f_1(\lambda/n^0, y^0) = \frac{(n^0y^0)n^{n+1}}{t(n^{n+1})} \lambda^{-(n^{n+1})-1} e^{-\frac{n^0y^0}{\lambda}}
\]  

(15)

\[
f_2(\lambda/n^0, \bar{y}^0) = \frac{(n^0y^0)n^{n+1}}{t(n^{n+1})} \lambda^{-(n^{n+1})-1} e^{-\frac{n^0y^0}{\lambda}}
\]  

(16)

\[
f_3(\lambda/\bar{n}^0, y^0) = \frac{(\bar{n}^0y^0)n^{n+1}}{t(n^{n+1})} \lambda^{-(n^{n+1})-1} e^{-\frac{n^0y^0}{\lambda}}
\]  

(17)

\[
f_4(\lambda/\bar{n}^0, \bar{y}^0) = \frac{(\bar{n}^0y^0)n^{n+1}}{t(n^{n+1})} \lambda^{-(n^{n+1})-1} e^{-\frac{n^0y^0}{\lambda}}
\]  

(18)

\( n^0 \): lower, \( \bar{n}^0 \): upper
\( \bar{y}^0 \): upper
\( y^0 \): lower

The equations (15)-(18) represent a set of prior distributions & a set of posterior distributions is obtained as shown below:

\[
f_1(\lambda \mid t) = \frac{(n^0 y^0 + \tau(t))n^0 + n + 1}{r(n^0 + n + 1)} \lambda^{-(n^0 + n + 1)-1} e^{-\frac{(n^0 y^0 + \tau(t))}{\lambda}} \tag{19}
\]

\[
f_2(\lambda \mid t) = \frac{(n^0 y^0 + \tau(t))n^0 + n + 1}{r(n^0 + n + 1)} \lambda^{-(n^0 + n + 1)-1} e^{-\frac{(n^0 y^0 + \tau(t))}{\lambda}} \tag{20}
\]

\[
f_3(\lambda \mid t) = \frac{(n^0 y^0 + \tau(t))n^0 + n + 1}{r(n^0 + n + 1)} \lambda^{-(n^0 + n + 1)-1} e^{-\frac{(n^0 y^0 + \tau(t))}{\lambda}} \tag{21}
\]

\[
f_4(\lambda \mid t) = \frac{(n^0 y^0 + \tau(t))n^0 + n + 1}{r(n^0 + n + 1)} \lambda^{-(n^0 + n + 1)-1} e^{-\frac{(n^0 y^0 + \tau(t))}{\lambda}} \tag{22}
\]

The equations (19) - (22) represent a set of posterior distributions & after taking the averages for those posterior distributions we get the iLuck-Model as shown below:

\[
\bar{y}^n = lower(y^n) = \begin{cases} 
\frac{n^0 y^0 + \tau(x)}{n^0 + n} & \text{if } \bar{\tau}(x) \geq y^0 \\
\frac{n^0 y^0 + \tau(x)}{n^0 + n} & \text{if } \bar{\tau}(x) < y^0
\end{cases}
\]

\[
\bar{y}^n = upper(y^n) = \begin{cases} 
\frac{n^0 y^0 + \tau(x)}{n^0 + n} & \text{if } \bar{\tau}(x) \leq \bar{y}^0 \\
\frac{n^0 y^0 + \tau(x)}{n^0 + n} & \text{if } \bar{\tau}(x) > \bar{y}^0
\end{cases}
\]

The posterior distribution will be final & as follows: (what does that mean)

\[
f(\lambda \mid n^m, y^m) = \frac{(n^m y^m n^m + 1)}{r(n^m + 1)} \lambda^{-(n^m + 1)-1} e^{-\frac{n^m y^m}{\lambda}} \tag{23}
\]

\[
n^m = \frac{lower(n^n) + upper(n^n)}{2}, \quad y^m = \frac{lower(y^n) + upper(y^n)}{2}
\]

Robust Bayesian To Estimate The Scale Parameter \( \lambda \)

From equation (23), the Bayesian estimator can be obtained for scale parameter \( \lambda \) under quadratic loss function which is the mean of the posterior distribution as shown below:

\[
\hat{\lambda}_{Rob} = \frac{n^m y^m}{n^m} \tag{24}
\]

Robust Bayesian To Estimate The Survival Function
From equation (23), the Bayesian estimator can be obtained for survival function under quadratic loss function which is the mean of the posterior distribution as shown below:

\[
\hat{S}_{\text{Rob}}(t) = \left( \frac{n_{my}^m}{n_{my}^m + t^m} \right)^{n_{m+1}}
\]  

(25)

**Steps of the simulation experiment**

The program was written using R & according to the following steps:

1. **The first step**
   - This step is one of the basic steps in which the default values are chosen as in the following steps: Different default values were chosen for scale parameter $\lambda$ and shape parameter $\beta$. The prior distribution parameters (a, b).
   - Three different samples were selected as follows:
     - $n$: 10, 20, 40
   - The frequency of the experiment was equal to (1000).

2. **The second step**
   - At this step, data is generated according to the following steps:
     - Generate the random variable $U_i$ that follows the uniform distribution:
       
       
       $U = R$
       
       Where:
       
       $U_i \sim U(0,1), \quad i = 1,2, \ldots, n$
       
     - Random variable $U$ is a random variable that describes a model under study using a statistical mathematical method. This method is used to generate various random variables that follows the various probability distributions. This method is characterized by its ease & efficiency:
       
       $u = F(t)$
       
       $t = F^{-1}(u)$
       
     - The random variable that follows the Weibull distribution is generated based on the above steps & is as follows:
       
       $t = e^{[\log(-\lambda \cdot \log(1-u))]}/\beta$

3. **Third Step**
   - The survival function and the scale parameter are estimated according to the Bayesian method & the robust Bayesian method.

4. **The fourth step**
The estimation methods are compared by using the following measures:

\[
\text{IMSE}(\hat{\lambda}) = \frac{1}{r} \sum_{i=1}^{r} (\hat{\lambda} - \lambda)^2
\]

\[
\text{IMSE}[\hat{S}(t)] = \frac{1}{r} \sum_{i=1}^{r} \left( \frac{1}{n_t} \sum_{j=1}^{n_t} [\hat{S}_i(t_j) - S(t_j)]^2 \right)
\]

Where:

r: Represents the frequency of experiment.

\( n_t \): Represents the sample size for each experiment \((t_i)\)

The simulation results will then be analyzed to estimate the scale parameter \(\lambda\) & the survival function of the Weibull distribution & according to the following tables as follows:

### Table (1) Integrated mean square error (IMSE) for the scale parameter \(\lambda\) under prior data unconflict

<table>
<thead>
<tr>
<th>(\beta=2)</th>
<th>(n^0)</th>
<th>(\lambda)</th>
<th>n</th>
<th>Regular Bayes</th>
<th>Robust Bayes</th>
<th>s.d prior</th>
<th>s.d posterior</th>
<th>best</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower 2</td>
<td>upper</td>
<td>10</td>
<td>0.182031</td>
<td>0.434197</td>
<td>3.181981</td>
<td>4.124883</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>0.169029</td>
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<td>0.141357</td>
<td>0.204936</td>
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</tr>
<tr>
<td>Lower 1.5</td>
<td>2.5</td>
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<td>0.345058</td>
<td>0.739248</td>
<td>5.656854</td>
<td>7.440964</td>
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### Table (1) Integrated mean square error (IMSE) for the scale parameter \(\lambda\) under prior data unconflict

<table>
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<th>(n^0)</th>
<th>(\lambda)</th>
<th>n</th>
<th>Regular Bayes</th>
<th>Robust Bayes</th>
<th>s.d prior</th>
<th>s.d posterior</th>
<th>best</th>
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<td>upper</td>
<td>10</td>
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<tr>
<td>Lower 2</td>
<td>3</td>
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</table>
Table (1) Integrated mean square error (IMSE) for the scale parameter $\lambda$ under prior data unconflict

<table>
<thead>
<tr>
<th>$\beta=4$</th>
<th>Lower</th>
<th>Upper</th>
<th>$n^0$</th>
<th>$\lambda$</th>
<th>n</th>
<th>Regular</th>
<th>Bayes</th>
<th>Robust</th>
<th>Bayes</th>
<th>s.d prior</th>
<th>s.d posterior</th>
<th>best</th>
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<tbody>
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<td>1.5</td>
<td>10</td>
<td>0.005336</td>
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Table (2) Integrated mean square error (IMSE) for the survival function under prior data unconflict

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**Table (3) Integrated mean square error (IMSE) for the scale parameter $\lambda$ under prior data conflict**

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### Table (3) Integrated mean square error (IMSE) for the scale parameter \( \lambda \) under prior data conflict

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Table (4) Integrated mean square error (IMSE) for the survival function under prior data conflict

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Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Administration & Economic and all experiments were carried out in accordance with approved guidelines.

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conflict» Bayesian Analysis, 2006;1:893–914.
Phenotypic detection of AmpC β-lactamase in *Pseudomonas aerogenosa* isolated from Burns and Wounds in Al-Rumetha Hospitals

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**Abstract**

Ampicillin hydrolyzing class C β-lactamase (AmpC) producing *Pseudomonas aeruginosa* has been recognized to be a serious opportunistic infection and due to the wide ability to create β-lactamases made this bacteria resistance to many antibiotics. The results of the current study showed that 36 (27.7%) isolates of *P. aeruginosa* from 130 isolates examined for cefoxitin susceptibility by disk diffusion method. Out of thirty six *P. aeruginosa* isolates, 29 (80.6%) were cefoxitin resistant. The modified three dimension test and AmpC disk test were used confirm the production AmpC β-lactamase in all cefoxitin resistant isolates, only 23 (79.3%) isolates were phenotypically confirmed to produce AmpC beta-lactamase. The result of this study showed that 12/36 (33.3%) isolates were positive with blaAmpC gene by conventional PCR technique.

**Keywords:** *Pseudomonas aeruginosa, AmpC, MTDT, Cefoxitin*

**Introduction**

*Pseudomonas aeruginosa* is a responsible for opportunistic diseases which commonly causes infections in burned patients and is mostly resistant to varied antimicrobial agents, also this microorganism is leading to nosocomial infection among immunocompromised patients suffering from burn, cystic fibrosis, AIDS and cancer. Commonly *P. aeruginosa* infections have been treated by fluoroquinolones, carbapenems, aminoglycosides and cephalosporins antibiotics. Nevertheless, resistance rate of *P. aeruginosa* to beta lactams, aminoglycosides, quinolones and carbapenems has been recorded from several countries. *Pseudomonas aeruginosa* producing of AmpC enzyme pose a threat to public health and demands the need to detect by phenotypic methods, the occurrence of AmpC β-lactamases from various clinical and environmental isolates due to the fact that antibiotic resistance is an growing problem in hospitals in a worldwide. Cephalosporinases such as AmpC β-lactamases are clinically important encoded on the chromosomes of the Enterobacteriaceae and a limited other organisms such as *Pseudomonas aeruginosa* where they responsible of resistance to penicillins, cefazolin, cephalexin, cefoxitin, and β-lactamase inhibitor-β-lactam combinations. Resistance to multiple drugs in *Pseudomonas aeruginosa* is commonly the result of mixture of many mechanisms such as metallo β-lactamases (MBLs), AmpC β-lactamases, extended spectrum β-lactamases (ESBLs), excessive expression of efflux pump and modifications of active site or outer membrane. Hydrolytic enzymes such as AmpC β-lactamases cleave the β-lactam ring and confer bacterial resistance to β-lactam antibiotics. MBLs and AmpC beta lactamases classified by Ambler as class B and class C respectively. Biotyping and serotyping are phenotypic method while plasmid profile analysis and PCR are molecular methods used for diagnostic purpose. Genes responsible for the beta-lactamase production in *P. aeruginosa* are typically resides either on plasmids or on the chromosome with the possible to exchange between bacterial populations.

**Materials and Method**

**Sample Collection**

A total of 130 clinical samples of *P. aeruginosa* from Al-Rumetha hospitals in Al-Muthanna province during the period of five months. Out of these, 60 wound and 70 burn swabs. Burn swabs were taken from the center of burn and wound lesion, depending on Collee *et al.* a dry swab must first be soaked with a little amount of Brain-Heart Infusion broth, then organisms...
were diagnosed using standard biochemical tests such as ability to produce oxidase, pigment formation, Gram staining, oxidation of glucose, growth at 42°C, gas and acid production on triple sugar iron agar as well as the samples were directly inoculated in CHROM agar™ Pseudomonas, blood agar, MacConkey agar, Nutrient agar and incubated for 24hr and at 37°C under aerobic conditions.

**Antibiogram:**

Antibiotic susceptibility of the all isolates were determined by Kirby-Bauer disk diffusion method according to CLSI recommendations. The antibiotics used comprise disks (Himedia, India): Ceftazidime (CEF, 30 μg), Imipenem (IMP, 10 μg), Meropenem (MEM, 10 μg), Cefotaxime (CTX, 30 μg), Amoxicillin/Clavulanic acid (AMC, 20/10 μg), Amikacin (AK, 10 μg), Erythromycin (E, 10 μg), Piperacillin (PI, 10 μg), Chloramphenicol (CHL, 30 μg), Cefoxitin (30 μg), Aztreonam (ATM, 10 μg), Rifampicin (R, 5 μg), Ciprofloxacin (5 μg), Tetracycline (30 μg). Moreover, different class of antimicrobials used by VITEK2 automatic system for confirmatory susceptibility tests of isolates.

**Detection of AmpC β-lactamases**

The ability of *Pseudomonas aeruginosa* isolates to produce AmpC enzyme was screened by testing cefoxitin susceptibility. The isolates that exposed resistance to the antimicrobial activity of cefoxitin were supposed initially to produce AmpC enzyme when their inhibition zone diameter ≤ 18 mm; and this acceptable phenotypic confirmation.

**Modified three dimensional test (MTDT):**

According to Manchanda and Singh and Parveen et al. This test was accomplished.

**AmpC Disk Test:**

This test also carried out as recommended by Parveen et al. Basak et al.

**Molecular detection of AmpC –lactamase:**

**DNA extraction**

The method of extraction Plasmid DNA was done by using the genomic DNA Mini kit, based on the manufacturer’s instructions (Geneaid, Korea).

**Determination of DNA product and purity:**

The use of nanodrop spectrophotometer for purpose of genomic DNA checking by measurement of the concentration and purity of extracted genomic DNA via evaluation the absorbance at (260 /280 nm).

**Preparing the Primers suspension:**

The whole volume of the PCR mixture set up in 20 μl includes 5 μl of lyophilized AccuPower® PCR PreMix (Bioneer, Korea), 10 pico/μl of each specific primer for target gene and 5μl of *P. aeruginosa* DNA template, so as according to presented in (table1) the amplification conditions started with thermocycler program.

**Table 1. The amplification conditions of PCR thermocycler for blaAmpC gene.**

<table>
<thead>
<tr>
<th>Steps</th>
<th>Temperature oC</th>
<th>Time</th>
<th>Cycle number</th>
</tr>
</thead>
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<td>Initial Denaturation</td>
<td>94</td>
<td>30sec</td>
<td></td>
</tr>
<tr>
<td>Denaturation</td>
<td>94</td>
<td>30 sec</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
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<td>1 min</td>
<td>35</td>
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<tr>
<td>Extension</td>
<td>72</td>
<td>1 min</td>
<td></td>
</tr>
<tr>
<td>Final extension</td>
<td>72</td>
<td>10 min</td>
<td></td>
</tr>
</tbody>
</table>

**Results**

This study showed that out of the One hundred and thirty samples were taken from Al-Rumetha hospitals, 36 (27.7%) *P. aeruginosa* isolates were diagnosed. The frequency of *P. aeruginosa* isolates from the wound and burn swab samples presented in (Fig.1). The highest *P. aeruginosa* percentage occurred in the burn 23(32.9%) followed by wound infections 13 (21.7%)(p≤ 0.05).
All the P. aeruginosa isolates were tested for various antibacterial agents to determine the resistant rate which include (Ceftazidime, Imipenem, Meropenem, Cefotaxime, Amoxicillin/Clavulanic acid, Amikacin, Erythromycin, Piperacillin, Chloramphenicol, Cefoxitin, Aztreonam, Rifampicin, Ciprofloxacin and Tetracycline). Fig. (2) show that the isolates were diverse in susceptibility to antimicrobial agents (Chloramphenicol 100%, Piperacillin 100%, Rifampicin 94.4% Erythromycin 94.4%, Ceftazidime 94.4%, Cefotaxime 94.4%, Cefoxitin 80.6%, Amoxicillin/Clavulanic acid 75%, Aztreonam 61.1% Ciprofloxacin 55.6%, Amikacin 47.2%, Tetracycline 41.7% Meropenem 27.8%, Imipenem 19.4%). The isolates that resist three classes of antibiotics or more are considered multidrug resistant.

Two methods were used to check of production AmpC β-lactamase among cefoxitin resistance P. aeruginosa isolates, of the 36 P. aeruginosa isolates, 29(80.6%) were cefoxitin resistant by the disk diffusion method and initially regarded as AmpC β-lactamase producers Fig.3.
The modified three dimension test was also used to confirm the production AmpC β-lactamase in all cefoxitin resistant isolates, only 23(79.3%) isolates were phenotypically confirmed to produce AmpC beta-lactamase. However, there is another method used to detect production AmpC β-lactamase among cefoxitin resistant P. aeruginosa isolates, this test is AmpC disk test and 23(79.3%) out of the 36 P. aeruginosa isolates were positive for AmpC beta-lactamase as demonstrated in (Table 2).

**Table (2): AmpC β-lactamase production among P. aeruginosa isolates.**

<table>
<thead>
<tr>
<th>Cefoxitin susceptibility of isolates</th>
<th>No. (%) of phenotypic AmpC β-lactamase producer isolates</th>
<th>No. (%) of AmpC- negative isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MTDT AmpC Disk Test</td>
<td></td>
</tr>
<tr>
<td>Resistant (n=29)</td>
<td>23(79.3%)</td>
<td>6(20.7%)</td>
</tr>
<tr>
<td>Susceptible (n=7)</td>
<td>0(0%)</td>
<td>0(100%)</td>
</tr>
<tr>
<td>Total (n=36)</td>
<td>23(63.9%)</td>
<td>6(16.7%)</td>
</tr>
</tbody>
</table>

L.S.D. 0.05 of isolate susceptibility = 2.987 methods= 1.547 Interaction=3.656

Molecular detection of AmpC β-lactamase production among the P. aeruginosa isolates 36(27.7%) with blaAmpC gene by PCR technique. This result showed that 12/36 (33.3%) isolates were positive with blaAmpC gene as presented in Fig. 4 and 5.
Discussion

The present study showed that the highest rate of *P. aeruginosa* occurred in the burn infections 23(32.9%) followed by wound infections 13 (21.7%)(p≤ 0.05). In Al-Muthanna, AL-Aaajipi 19 found that the *P. aeruginosa* prevalence rate was (44.64%) from the burn which is higher than our study. The current results are agreement with Azeez and Bakr 20 who found the highest *P. aeruginosa* prevalence rate occurred in the burn(40%) followed by wound infections (17.5%). As well as, Nasih et al. [2014] recorded (17%) *P. aeruginosa* from the patients. Another study showed that (38%) *P. aeruginosa* isolates among 100 burn samples in Baghdad hospitals 21. *P. aeruginosa* is currently one of the major bacterial pathogens responsible for nosocomial infections and due to resistance to various antibiotics the treatment of these infections has become very difficult. The results described in (Fig.2) demonstrates that the isolates were (100%) resistant to Chloramphenicol and Piperacillin respectively, similar studies were performed by other researcher Azeez and Bakr 20 showed that among 50
isolates of P. aeruginosa, 100% resist to Penicillin, Lincomycin, Vancomycin, Piperacillin, Rifampicin, and Chloramphenicol, as well as Ali 2016 illustrated that out of the 60 P. aeruginosa isolates, 30% resist to fourteen various antibacterial agents but all isolates were resistant to G, L, CEF, ATM, PI, and CTX. The reasons of wide range of resistance is dependent upon excessive use or misuse of antibiotics, hospital setting, transmission of resistance genes among bacterial species. In this study, 94.4% of the isolates were resistant to Rifampicin, Erythromycin, Ceftazidime, and Cefotaxime, also 80.6% to cefoxitin, this resistance is due to the presence of other mechanisms such as AmpC type β-lactamases or MexAB-OprM Efflux Pumps. This resembles with the results of Chika et al. who reported P. aeruginosa isolates were resistant to Ceftazidime (92%) and cefoxitin (80%). Furthermore, the percentage of the susceptibility to Meropenem was 27.8% and Imipenem 19.4% therefore considered most effective antibiotics, this is agreed with Azeez and Bakr who found the lowest resistance ratio was for Imipenem 4% and Meropenem 20%. Therefore due to the sensitivity of the bacterial isolates to imipenem and meropenem, making them the best therapeutic option. This sensitivity is due to the patients infected with bacterial isolates producing AmpC beta-lactamases using imipenem as last option. But this results mismatched with Salimi and Eftekhari, 2013 who showed P. aeruginosa was 93.20% resistant to Imipenem and 94.17% to Meropenem. The production of AmpC beta-lactamase is one among numerous resistance mechanisms discovered in P. aeruginosa from both the community and hospital environment is fast becoming an international risk and the appearance and development of these multidrug resistant organisms is a warning to existing drugs when antibiotics are over prescribed so as when people lack of interest in personal hygiene this leads to resistance to antibiotics. The primary screening test of the molecular class C β-lactamases (AmpC) used in the laboratory is modified three-dimensional test, this results revealed 23(79.3%) isolates were phenotypically confirmed to produce AmpC beta-lactamase in this test, all the isolates were resistant to Cefoxitin while the remaining 6(20.7%) cefoxitin resistant isolates were negative for molecular class C β-lactamase due to the reduction of antibiotic penetration across porins or these isolates probably have genes for plasmid-mediated and/or chromosomal AmpC beta-lactamase production but silent genes or not encoded in these isolates.

**Conclusion**

The best way to diagnose AmpC β-lactamase is PCR technique. The current study showed high rates of resistance to Phenics, Penicillins, Ansamycins, Macrolides, Cephalosporin III and Cephamycins in P. aeruginosa isolated from patient in Al-Rumetha Hospitals, Iraq, which could help many doctors prescribe the best antibiotics for treatment of P. aerogenosa infections. Furthermore, the current study also showed most of these isolates had ampC genes and there was important correlation between resistant to β-lactam antibiotics and existence of ampC gene.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under Biology Dep./ Education for Pure Science College/Al-Muthanna University and all experiments were carried out in accordance with approved guidelines.

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Assessment Knowledge of Thalassemia Care Giver in Kirkuk City

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College of Nursing / University of Kirkuk, Iraq

Abstract

Thalassemia is an inherited blood disorder, which is described by diminished synthesis or lack of globin, this synthetic defect leads to the formation of fragile abnormal red blood cells (RBC), which can be easily hemolyzed, leading to chronic anemia. Quantitative design descriptive study was used to assess Knowledge Thalassemia Care givers in Kirkuk City. A non–probability / Convenience sampling technique was applied in the present study, (30) Thalassemia Care givers were participate in the study sample (Azadi Teaching Hospital) in Kirkuk City. A questionnaire was designed and constructed by researchers to measure the variables underlying the present study. Data were obtained directly by the researchers through interview technique. Content validity of the instrument was determined through the of panels which involve (5) experts to investigate the clarity. The findings in general indicated that Majority of the sample In general has good Knowledge about Thalassemia it was recorded (74.8%) were answered “I Know.

Keywords: Assessment Knowledge of Thalassemia care giver in Kirkuk city

Introduction

Thalassemia is an inherited blood disorder, which is described by diminished synthesis or lack of globin, this synthetic defect leads to the formation of fragile abnormal red blood cells (RBC), which can be easily hemolyzed, leading to chronic anemia. This disorder is exceptionally pervasive among children in the Middle East, Mediterranean region, and South Asia, though, only a few studies on pediatric quality of life have been available from those areas. The management of thalassemia contains regular blood transfusion, iron chelation treatment, and suitable management of comorbidities. These modalities led to an increase in the life expectancy of thalassemic children, therefore, stressing the significance of keeping up the personal satisfaction in kids with thalassemia. Thalassemia has a negative influence on the physical functioning of children and adults. It can likewise influence social connections and emotional well-being, in the long run prompting poor school execution and general disability in the wellbeing related personal satisfaction. It has been accounted for that around 80% of thalassemic patients have psychiatric issues, it was accounted for already that enthusiastic pain and sickness trouble affect the personal satisfaction of patients as gloom and uneasiness related indications; nonetheless, culture and the sort of treatment did not. Pediatric personal satisfaction estimation is an instrument that is utilized to evaluate the impact of ailment on a patient’s prosperity, distinctive perspectives are canvassed in pediatric personal satisfaction, including physical, mental, and social working as different components can influence personal satisfaction.

General objective:

“Assessment Knowledge Thalassemia Care givers in Kirkuk City”

1.2 Specific objectives:

1- To identify socio demographic characteristics of the caregiver’s (age, gender, educational level, occupation, marital status).

2- To identify caregiver’s knowledge regarding thalassemia.

Alpha thalassemia

Each human diploid cell contains four copies of...
the alpha-globin gene, located on chromosome, Alpha thalassemia is the result of reduction in the synthesis of the alpha globin chains and a form of thalassemia involving the gene HbA1 and HbA2. Two main types of alpha thalassemia are described as alpha thalassemia major and hemoglobin H disease that Alpha thalassemia major is a very serious disease of severe anemia that begins even before birth, most affected babies do not survive full gestation or die shortly after birth. Hemoglobin H disease is milder than beta thalassemia and does not generally require transfusion therapy.

**Beta Thalassemia**

The most familiar type of thalassemia is beta thalassemia. Thalassemia was first recognized clinically in 1925 when Thomas Cooley described a syndrome of anemia, splenomegaly, and bony deformities among Italian descents, Beta thalassemia or Cooley’s anemia is caused by a change in the gene for the beta globin component of hemoglobin. Beta thalassemia is caused by damaged or missing genes, thalassemia can be subjected to modification in the hemoglobin genes depending on the mild and mild side effects. Beta thalassemia patients is most found in people who are from Greek, Italian, African, or Asian origin especially India, Beta thalassemia major has begun since childhood and will last until the end of the life, the severe anemia can result in severe lethargy, paleness, and insufficient growth and development, Other characteristic physical complications such as heart problems and excessive liver and spleen growth which decrease life-expectancy, this condition is becoming more prevalent in the USA as a result of Asian immigration.

**Management of Thalassemia**

**Thalassemia minor**

Patients with thalassemia minor generally don’t require a particular treatment, educate patients that their condition is genetic and that doctors now and then mix up the turmoil for iron insufficiency, some pregnant patients with the beta thalassemia characteristic may create simultaneous iron inadequacy and serious sickness; they may require transfusion bolster on the off chance that they are not receptive to iron repletion modalities. (Karimi M., 2009)

**Thalassemia major**

The objective of long-term hypertransfusion bolster is to keep up the patient’s hemoglobin level at 9 - 10 g/dL, along these lines enhancing his or her feeling of prosperity while all the while smothering upgraded erythropoiesis, this methodology treats the weakness and smother endogenous erythropoiesis with the goal that extra medullary hematopoiesis and skeletal changes are stifled. Patients accepting long haul transfusion treatment additionally require iron chelation. (Fatimah Sharif Modawi., 2017).

Blood banking contemplations for these patients incorporate totally writing their erythrocytes for Rh and ABO antigens preceding the primary transfusion. This methodology helps future cross-coordinating procedures and limits the odds of all immunization. Transfusion of washed, leukocyte-poor red blood cells (RBCs) at roughly 8 - 15 mL RBCs for each kilogram (kg) of body weight more than 1 - 2 hours is suggested.

Erich-repointing (EPO) levels were higher in guys: 72 versus 52 mIU/mL (P = 0.006). The frequency of splenic tomy was higher in guys (61%, versus 40% in females; P = 0.031). Allogeneic hematopoietic transplantation can be remedial in some patients with thalassemia major, the early successful allogeneic stem cell transplant from an HLA-identical sibling donor was stated in 1982, an Italian group led by Lucarelli has the most experience with this procedure, this group’s research documented a 90% long-term survival rate in patients with favorable characteristics (young age, HLA match, no organ dysfunction), transplantation-related issues such as graft versus host disease, graft failure, chronic immunosuppressive treatment, and transplantation-related mortality must be cautiously considered earlier to proceeding with this method.

**Surgical Treatment**

Splenectomy is the vital surgical system utilized for
some patients with thalassemia, the spleen is known to contain a lot of the labile nontoxic iron (i.e. stockpiling capacity) got from sequestration of the discharged iron. The spleen additionally expands RBC decimation and iron conveyance, these realities ought to dependably be considered before the choice is made to continue with splenic tomy, what’s more, with late reports of venous thromboembolic events after splenic tomy, one ought to precisely consider the advantages and the dangers previously splenic tomy is supported, the spleen goes about as a store for nontoxic iron, along these lines shielding whatever remains of the body from this iron. Early evacuation of the spleen might be unsafe.

**Methodology**

This chapter deals with the presentation of the administrative arrangement, design of the study, setting of the sample, sample of the study, Instrument of the study, validity, pilot study, method of data collection, period of data collection statistical analysis and limitation of the study.

**Design of the Study:**

A descriptive study was carried out from February, 1st, 2019 to April, 15th, 2019 in order to achieve the objectives of the present study.

**Setting of the Study:**

The study was conducted in Azadi hospitals in Kirkuk city.

**Sample of the study:**

Non-probability sampling approach (purposive sample) consists of 62 nursing staff of premature units in Azadi hospitals.

**Criteria:**

**Inclusions:**

Only nursing staff who were working in the premature unit in the hospitals

**Instrument Construction:**

1. For the purpose of the present study, a questionnaire format was constructed to assessment of the nursing staff knowledge and practices regarding neonatal jaundice.

2. The questionnaire format was based on the review of literature and related previous studies. The questionnaire was designed in English and then translated into Arabic language. It was reviewed by supervisor and experts.

3. Formal consent was obtained from the nursing staff who agreed to participate in the study, the questionnaire was self-administer and took 10-15 minutes to be complete.

4. The study instrument of comprised of three parts, which was consisted the following variables:

1. **Part One/Socio-demographic characteristics**

   Include: Type of hospital, Sex, Educational level, Marital status, residence, Socio-economic status, years of experience in premature unit, participation in a training course, period of the training course and the place of the training.

2. **Part Two/Assessment of knowledge:**

   Include 20 items regarding knowledge of the sample. It has been consist of three scales as ‘Know’, ‘I didn’t know’ and ‘Uncertain’.

3. **Part Three/ Assessment of practice**

   Include 4 items regarding practices of the study sample.

**Validity of the study scale:**

To ensure the validity of the scale, method and procedure were proposed to be carried out during the study. Nine experts of different specialties related to the field of the present study were chosen to review face and content validity. They were asked to review the scale format for clarity and adequacy in order to achieve the present study objectives. Those experts were all faculty members from the College of Nursing/ University of Kirkuk, (82%) of them had agreed that the scales were clear, relevant and adequate. Certain modifications were employed based on the experts’ recommendations and suggestions.

**Method of Data Collection:**

Data were collected through self-administered technique, The investigators had demonstrated objectives and the significance of research and the benefit of the study to the participant. Verbal consent obtained from premature unit nursing staff. Each nurse
spends approximately (10-15 min) to respond to the questionnaire.

**Period of Data Collection:**

The data collection has been conducted during a period of two months extending from February 15th, 2019 to April 15th, 2019.

**Statistical Analysis**

Data were analyzed in several steps. First, descriptive statistics, which includes frequency and percentages, and Mean score. Data are prepared, organized and entered into the computer file; Statistical Package for Social Science (SPSS)(20 version) is used for data analysis at (P. value ≤ 0.05). Data are analyzed through the application of two approaches:

**Descriptive statistical data analysis:**

This approach is employed through:

- Frequency distribution
- Percentage (%)

\[
\% = \left( \frac{\text{Frequencies}}{\text{sample size}} \right) \times 100 \quad (32)
\]

- Mean of Scores

This computation is applied for the determination of item’s significance of the assessment tool relative to each aspect. Cut-off points are used for this determination low-significant, moderate significant, and highly significant.

\[
\text{M.S} = \frac{f_1 \times \text{score}_1}{n_1} + \frac{f_2 \times \text{score}_2}{n_2}
\]

It is computed as follows:

M.S = Mean of score

f = Frequency

n = Number of cases

**Rating and scoring of the scale:**

The knowledge and practices questionnaire items were rated and scored to items as, Know, I didn’t know and Uncertain. Data of the study were ordinal according to three levels scale which were scored for each level respectively.

**Result**

This chapter will present the research according to the statistical analysis for the data collection during the study period. Regarding to sex, (70%) of the participants were female, while only (30%) were male. According to the educational level (10%) of the participants were Illiterate, (26.7%) of them had write & read, (20%) of them were primary school, (23.3%) of them were secondary school, while (20%) were Institution. According to marital status, (80%) of study sample were married, while (13.3%) were single, while (6.7%) were separated. Regarding to Occupation of caregiver, (10%) were Governmental employee, (40%) were Non-governmental employee, and (50%) were Jobless. According to socio-economic status, (58.1%) had barely sufficient economic status and (27.4%) had sufficient economic status. Regarding to Age of caregiver, (26.6%) of participants had (≥ 19 years)’ experience, while (40%) of them had (20 – 29 Y) years of experience, while (30%) of them had (30 – 39 Y), while (3.4%) were (≤ 40 Y) years.

**Conclusion**

Thalassemia intermedia has a wide clinical spectrum, as some patients are completely asymptomatic until adult life whereas others present with the condition at 2 years of age and experience retarded growth and development. Many patients with thalassemia intermedia do not currently undergo transfusion therapy due to difficulties in deciding when to initiate therapy as well as the lack of a convenient and effective iron chelator. However, the availability of such a therapy may increase the use of transfusions in patients with thalassemia intermedia, allowing them to benefit from this therapeutic approach and avoid any subsequent clinical complications. As there are currently no clear guidelines for the management of thalassemia intermedia, in this paper, we present some recommendations based on a system-centered risk stratification model to help individualize patient treatment. All patients diagnosed to have thalassemia based on High Performance Liquid Chromatography (HPLC) will be included in the study. It includes β thalassemia trait, β thalassemia intermedia, β thalassemia major, α thalassemia, double heterozygous conditions like Beta thalassemia HbS, Beta thalassemia HbE and Beta thalassemia HbD.
Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under College of Nursing / University of Kirkuk, Iraq and all experiments were carried out in accordance with approved guidelines.

References


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Evaluation the Effect of Nano Yttrium Oxide Addition on the Mechanical Properties of Room Temperature-Vulcanized Maxillofacial Silicone Elastomers

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Abstract

Objective: This study aims to investigate the effects of yttrium oxide (Y$_2$O$_3$) nanopowder inclusion on the mechanical properties, including tear strength, tensile strength, elongation percentage, hardness, and surface roughness, of room temperature-vulcanized maxillofacial silicone elastomers.

Materials and Method: Y$_2$O$_3$ nanoparticles (30–45 nm) were added to VST50F platinum silicone elastomers in two weight percentages (1 and 1.5 wt%) as tested by the pilot study that showed superior results. A total of 120 specimens were qualified and divided into the control group and experimental groups (1 and 1.5 wt% Y$_2$O$_3$). Each group was subdivided into four identical subgroups in accordance with intentionally performed tests (tear strength, tensile strength and elongation percentage, shore A hardness, and surface roughness). For each subgroup, 10 specimens were used for each test. Data were statistically analyzed with significance level considered at $p<0.05$. Scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR) were also conducted.

Results and Discussion: SEM showed that NPs were distributed well within the silicon matrix. FTIR spectra proved no chemical reaction occurred between the Y$_2$O$_3$ NPs and VST50F silicone. Tear strength and hardness values significantly increased in the experimental groups compared with the control group. Tensile strength and surface roughness values increased non significantly. Elongation percentage values decreased non significantly.

Conclusion: Incorporating Y$_2$O$_3$ NPs into VST50F maxillofacial silicone improved the tear strength with slight increase in tensile strength. Hardness and surface roughness increased but within accepted clinical levels. Elongation percentage slightly decreased.

Keywords: maxillofacial silicone, Y$_2$O$_3$, nanoparticles, mechanical properties.

Introduction

Prosthetic rehabilitation may become unavoidable for multiple reasons because of the extent or site of facial defect following congenital anomaly, injury, or after excision of tumors. Materials such as ivory or metals were used in the past, but polymers such as silicone elastomers have recently become the most preferred for the construction of maxillofacial prostheses because of their chemical dormancy, endurance, easy usage, and biocompatibility. However, given their anticipated half-life and deterioration of mechanical properties and color stability, maxillofacial prostheses composed of silicone elastomers require refabrication. Factors that influence the mechanical propriety of silicone elastomers are the atomic density of polymer chains, cross-linking closeness, and presence of fillers. Throughout the evolution of the nanoparticle (NP) industry, NPs have...
merged into the polymer matrix to equip a modified polymer characterized by an augmented properties gained from the reinforcing effect of the nano-oxides.  

Considerable studies were operated to overcome the mechanical weaknesses of materials, such as reinforcing fillers. A previous study found that adding TiO$_2$, ZnO, and CeO$_2$ as nanofillers to A-2186 silicone in two weight percentages (2.0% and 2.5%) improves the overall mechanical properties (tear strength, tensile strength, and elongation percentage) of the material. Another study analyzed the influence of adding (nano ZnO, BaSO$_4$, and TiO$_2$ at 1 and 2 wt%) on the tear strength, hardness, and permanent deformation of silicone and recommended the use of ZnO NPs to reinforce (Silastic MDX4-4210) silicone. In general, a powerful interaction occurs at the interface between the organic polymeric matrix and the NPs and produces a reinforcing effect. In the present study, Y$_2$O$_3$ nanofillers were added to VST50F RTV maxillofacial silicone elastomer to enhance its mechanical properties.

**Materials and Method**

Y$_2$O$_3$ nanopowder (US Research Nanomaterials, Inc., USA) was added to VST50F room temperature-vulcanized maxillofacial silicone elastomers (Factor II, Inc., Lakeside, AZ, USA) to investigate its effect on some mechanical characteristics.

**Pilot study:** The best weight percentage of nano Y$_2$O$_3$ to improve the tear strength with minimal effect on the hardness of the silicone elastomer was selected. The most suitable weight percentages were 1 and 1.5 wt%.

**Study grouping:** A total of 120 specimens were fabricated and divided into the control group (silicone without nano Y$_2$O$_3$ addition referred to as group A) and experimental groups (with 1% Y$_2$O$_3$ addition referred to as group B and with 1.5% Y$_2$O$_3$ addition referred to as group C). Each group was subdivided into four identical subgroups in accordance with intentionally performed tests.

**Mold making:** An autoCAD (Computer Aided Design) software was used to design the dimensions of specimens, and then a Computer Numerical Control machine (JL-1612, Jinan Link Manufacture and Trading Co., Ltd., China) was used to obtain the matrix compartment of the mold where the material will be poured.

**Mixing base, catalyst, and nano Y$_2$O$_3$:** Y$_2$O$_3$ nanopowder was measured using a digital electronic balance with 0.000 accuracy (China) followed by adding the intended amount of silicone base to avoid the scattering of volatile nanopowder and subsequent weight measurement change. The silicone base was mixed with the nanopowder by using a vacuum mixer (Multivac 3, Degussa, Germany) for 10 min at 360 rpm to form the modified base. The first 3 min of mixing comprises no vacuum suction to preserve the nanopowder, whereas a vacuum pressure of -10 bar was applied in the next 7 min to remove air bubbles. To obtain a proper working time, we combined the accelerator at a base-to-catalyst ratio of 10:1 (according to the manufacturer instructions) with the modified silicone base for 5min with vacuum suction of -10 bar. The mixing was accomplished at a relative humidity (RH) of 50% ± 10% and temperature of 23 °C ± 2 °C.

**Pouring the material into the mold:** The mixture was poured on the acrylic mold in excess amount to reduce the percentage of air bubbles that may be formed on the top surface of the samples when the cover was placed. The cover was then applied and locked with screws, nuts, and G-clamps with a constant load application of 1 kg. The air bubbles on the specimen surface were removed by applying constant pressure until the material was completely cured. The material was poured under standard conditions (RH of 50% ± 10% and temperature of 23 °C ± 2 °C).

**Specimen storage, retrieval, and finishing:** The specimens were reserved in a storage box (for constant results, atmospheric conditions that might affect the silicone properties were controlled) for 16–72 h. Temperature must be within 20 °C – 25 °C, humidity should be within 50% – 60%, and care must be taken to avoid laying the specimens one over other. Specimens were protected from light exposure from vulcanization to testing (the minimum time for vulcanization is 16 h). After polymerization, the specimens were removed from the molds. The tested specimens must remain intact with well-defined borders, free from air bubbles and clear from irregularities on the surface and inside, particularly at the stress-loaded areas. Defects would cause the specimen to be discarded. Flashes of the specimens were removed with a scalpel and surgical blade.

**Mechanical testing procedures:**

A) **Tear strength:** Specimens were prepared and
tested in accordance with ASTM D624, 2013 (type C specimens) by using a universal testing machine (Laryee Technology Co., Ltd., China) running at a velocity of 500 mm/min, by which specimens were stretched until complete rupturing at the apex. The following equation was applied: Tear strength = f/d, where f is the highest force recorded to rupture the specimen at the apex (kN) and d is the specimen average thickness (m).

B) Hardness: Specimens were prepared and tested in accordance with ISO 7619-1, 2010 (square specimens with 25 mm length and width and 6 mm thickness). A Shore A hardness digital tester (HT-6510A, China) with a dull indenter having a diameter of 1.25 mm was used. The hardness value is the mean of five different measurements whose points lie at a distance of 6 mm from each other and from the border.

C) Tensile strength and elongation percentage: The specimens were prepared and tested in accordance with ISO 37, 2011 (Type 2) in a dumbbell shape by using a universal testing machine running at a velocity of (500 mm/min) by which the specimen will be stretched until complete rupturing at the thin portion. The tensile strength = F/A, where F expresses the highest force recorded to rupture the specimen at the thin portion (kN) and A expresses the specimen cross-sectional area (m²) (width x thickness). The elongation percentage can be calculated simultaneously where the specimen length at the beginning and the length at rupturing can be applied in the following equation:

\[ \text{Elongation percentage} = \left( \frac{L_b - L_o}{L_o} \right) \times 100 \]

D) Surface roughness: The specimens resemble those of hardness with dimensions (25 mm×25 mm×6 mm). A digital surface roughness tester (SRT-6200S, China) was used, and its diamond probe acts by moving on the specimen surface. The average of three readings represents the surface roughness value of that specimen.

Additional tests:

A) Scanning electron microscopy (SEM): An electron microscope (Inspect S50, FEI company, Nederland) was used to check the distribution of the NPs within the silicon matrix.

B) Fourier transform infrared spectroscopy (FTIR): A Fourier transform infrared spectrometer (Tensor 27, Bruker, Germany) was used to confirm whether or not a chemical reaction had occurred between the Y₂O₃ nanopowder and the silicone elastomer.

Statistical analysis: The analysis was accomplished by an SPSS software with significance level set at \( p < 0.05 \) and includes

(1) Descriptive statistics: bar chart graphics.

(2) Inferential statistics: ANOVA and post-hoc tests such as Levene, Tukey’s HSD, and Dunnett T3 tests.

Findings

SEM and FTIR examinations: SEM images show the well dispersion of the Y₂O₃ NPs within the silicone matrix (Figure 1A and B). From the chemical point of view as shown in the FTIR spectrum, no interaction occurred between Y₂O₃ nanopowder and VST50F maxillofacial silicone.
**Tear strength:** The tear strength significantly increased after the addition of Y$_2$O$_3$ ($p < 0.05$) with groups B and C exhibiting higher means than group A (Table 1).

### Table 1: Descriptive statistics, one way ANOVA and Tukey's HSD of tear strength.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>±SD</th>
<th>F</th>
<th>p value</th>
<th>Groups</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>27.5</td>
<td>30.4</td>
<td>28.5</td>
<td>.786</td>
<td></td>
<td>11.912</td>
<td>A B</td>
<td>.025 S</td>
</tr>
<tr>
<td>B</td>
<td>28.9</td>
<td>34.8</td>
<td>30.5</td>
<td>1.944</td>
<td>0.000</td>
<td>[HS]</td>
<td>A C</td>
<td>.000 HS</td>
</tr>
<tr>
<td>C</td>
<td>29.8</td>
<td>35.5</td>
<td>31.98</td>
<td>1.813</td>
<td></td>
<td></td>
<td>B C</td>
<td>.116 NS</td>
</tr>
</tbody>
</table>

Levene statistics=2.645, p value=0.089[NS]

The highly significant increase in the tear strength may be related to the physically trapped networks formed due to the 3D cumulative capability of the NPs that creates networks of fillers within the polymer matrix. These trapped networks participate in hindering the movement of the polymer segment against the NP surface and preventing the movement of other polymer chains against each other, thereby changing the overall network density and markedly increasing polymer stiffness$^{22}$.

**Hardness:** The hardness significantly increased after the addition of Y$_2$O$_3$ ($p < 0.05$) with groups B and C exhibiting higher means than group A (Table 2).

### Table 2: Descriptive statistics, one way ANOVA and Dunnett's T3 of hardness.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>±SD</th>
<th>F</th>
<th>p value</th>
<th>Groups</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>26.8</td>
<td>27.2</td>
<td>26.89</td>
<td>.137</td>
<td></td>
<td>86.669</td>
<td>A B</td>
<td>.000 HS</td>
</tr>
<tr>
<td>B</td>
<td>27.9</td>
<td>29.3</td>
<td>28.43</td>
<td>.488</td>
<td>.000</td>
<td>[HS]</td>
<td>A C</td>
<td>.000 HS</td>
</tr>
<tr>
<td>C</td>
<td>28.5</td>
<td>29.8</td>
<td>29.13</td>
<td>.445</td>
<td></td>
<td></td>
<td>B C</td>
<td>.011 S</td>
</tr>
</tbody>
</table>

Levene statistics=7.860, p value=0.002[HS]

The highly significant increase in hardness may result from the filler-to-filler binding caused by increased concentration of nanofillers occupying the areas between polymer chains rendering them lesser. This phenomenon increases the rigidity of the polymer to resist penetration$^{23}$ and may also explain the significant difference between the two experimental groups where the addition of 1.5% Y$_2$O$_3$ increases the hardness more than the addition of 1% Y$_2$O$_3$.

**Tensile strength:** The tensile strength increased non significantly after the addition of Y$_2$O$_3$ ($p > 0.05$) with groups B and C exhibited higher means than group A (Table 3).

### Table 3: Descriptive statistics and one way ANOVA of tensile strength.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>±SD</th>
<th>F</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4.660</td>
<td>6.650</td>
<td>5.785</td>
<td>.615</td>
<td>.175</td>
<td>.841[NS]</td>
</tr>
<tr>
<td>B</td>
<td>4.840</td>
<td>6.420</td>
<td>5.859</td>
<td>.574</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>4.930</td>
<td>6.760</td>
<td>5.942</td>
<td>.594</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The slight increase in tensile strength can be attributed to the physical interaction occurring between the NPs and the silicone matrix as polymer chains and the filler particles within slide over each other as a result of the applied tensile forces; the nanofiller presence prevents breakage of the polymer chains 24.

**Elongation percentage:** The elongation percentage decreased non significantly after the addition of Y$_2$O$_3$ ($p > 0.05$) with group A had mean value higher than group B and C (Table 4).

**Table 4: Descriptive statistics and one way ANOVA of elongation percentage.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean ±SD</th>
<th>F</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5.570</td>
<td>6.940</td>
<td>6.365 .465</td>
<td>1.390</td>
<td>.266[NS]</td>
</tr>
<tr>
<td>B</td>
<td>5.270</td>
<td>6.830</td>
<td>6.115 .511</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>5.130</td>
<td>6.940</td>
<td>5.973 .610</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The slight decrease in elongation percentages might be caused by the disruption of the proper orientation and flow of molecular chains, providing the matrix with additional restraint and causing earlier failure at several areas and eventually less elongation percentage values 25.

**Surface roughness:** The surface roughness increased non significantly after the addition of Y$_2$O$_3$ ($p > 0.05$) with groups B and C exhibited higher means than group A (Table 5).

**Table 5: Descriptive statistics and one way ANOVA of surface roughness.**

<table>
<thead>
<tr>
<th>Surface roughness(µm)</th>
<th>F</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>.25</td>
<td>.47</td>
</tr>
<tr>
<td>B</td>
<td>.29</td>
<td>.49</td>
</tr>
<tr>
<td>C</td>
<td>.29</td>
<td>.44</td>
</tr>
</tbody>
</table>

The randomly arranged NPs within the silicon matrix and on the specimen surface during preparation might increase the mean value of surface roughness after the addition of Y$_2$O$_3$ nanopowder 26.

**Conclusion**

No chemical interaction occurred between the Y$_2$O$_3$ NPs and VST50F silicone. The addition of Y$_2$O$_3$ NPs to VST50F maxillofacial silicone improved the tear strength and slightly increased the tensile strength. The hardness also increased within the accepted clinical range, and the surface roughness slightly increased. The elongation percentage slightly decreased.

**Conflict of Interest:** We hereby declare no conflict of interest.

**Source of Funding:** Entirely self-funded.

**Ethical Clearance:** In vitro study.
References


26. Leny M. Development of Elastomeric Hybrid Composite Based on Synthesized Nanosilica and short Nylon Fiber [PhD thesis]. Cochin, Cochin University of Science and Technology; 2009.
Immunohistochemical Correlates of Epidermal Growth Factor Receptor Mutations in Lung Adenocarcinoma in Iraq Patient

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Department of Biology, Faculty of Science, University of Kufa/Iraq

Abstract

We aimed to investigate the Detection of epidermal growth factor receptor (EGFR) mutations in lung adenocarcinoma by Immunohistochemical and correlation with Clinicopathological features. We used Fifty tissue blocks embedded in paraffin wax were obtained from 50 patient (16 female and 34 males) suffering from lung adenocarcinoma, while controls were 30 samples from healthy, Histopathological examination, Study by (H and E stain) and detection of Mutation in EGFR(exon19) by RFLP-PCR, detection of protein expression EGFR by immunohistochemical method. Our results have confirmed the mean age of patients group was 51.84 +/-15.70 years and the median was 54, performed study the non correlation between stage of disease and age of patients. site of tumor was right sided of lung tumor, accounting for 58%. Distribution of patients according to grade of tumor was, The results of this study showed non-significant correlation between age and grade, results of this study showed significant difference in EGFR Positive between patients and control healthy in Imunohistochemical, had found as not significant with age, gender, sit of tumor, grade, stage, in Immunohistochemical method of EGFR Expression. while Gene EGFR deletion of exon 19 were analyzed using mutant-enriched polymerase chain reaction (PCR) was not detected in all patients and control.

Keywords: Epidermal growth factor receptor (EGFR); Immunohistochemical; lung adenocarcinoma; prognostic factors.

Introduction

Lung cancer is currently the most frequent cause of major cancer incidence and mortality worldwide.1 Two main histological categories are included: small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC). NSCLC comprises about 80% of all diagnosed lung cancer, and lung adenocarcinoma is the most common subtype of NSCLC.2 This fact is due to several reasons, including increases in life expectancy, higher pregnancy age, poor nutrition, sedentary lifestyle and cigarette smoking.3 The use of tobacco cigarettes is the single greatest risk factor in the development of lung cancer, with up to 90% of lung cancers attributed to smoking.4

The EGFR (also referred to as: ERBB, ERBB1, HER1) gene is located on the short (p arm of chromosome 7 at position 12 (cytogenetic chr band 7p12.1).5 Among its main exons (n=28), exons 18/19/20/21 are critical for oncologists handling lung carcinoma patients. The most common mutations are a deletion in exon 19 and L858R point mutation,(6,7,8) clustered around the amino-acid residues 747–750(delE746-A750,delL747-T751insS, and delL747-P753insS) and a specific exon21 point mutation L858R(leucine to arginine [L858R] and leucine to glutamine [L861Q]) have been reported to comprise up to 90% of all activating EGFR mutations.(9,10) Mutations, amplifications or miss regulations of EGFR or family members are implicated in about 30% of all epithelial cancers lung epithelial malignancies constitute the diagnosis attributed to the majority of patients suffering from lung cancer (about 85% of all pathologically defined lung cancer cases).11 Immunohistochemical techniques detect antigens in tissue sections by means of immunological and chemical reactions.12 while PCR is a method for the in-vitro enzymatic synthesis of specific DNA sequences using two synthetic oligonucleotides primers that each hybridizes to opposite strands and flanks the region of interest in the target DNA, permitting the amplification of small amounts of genetic material13.
The aims of our study were to fined correlation between the IHC expression and presence of EGFR mutations with study of clinicopathological (age, sex, stag, grade) in the Patients.

**Patients and Methods**

This study was done in the department of Biology Lab, Faculty of Science, Kufa University during the period from April 2018 to May 2019. A total of 80 samples, Fifty tissue blocks embedded in paraffin wax were obtained from 50 patients (16 female and 34 males) suffering from lung adenocarcinoma, while controls were 30 samples from healthy volunteers, our study included the following:

A. **Histopathological Examination Study:**

Sections were obtained with 5 μm-thick from paraffin embedded tissues, these sections were stained by using Haematoxylin and Eosin staining method (H and E stain).

B. **Immunohistocemical staining.**

For IHC staining, 5-micrometer-thick sections were deparaffinized and placed on +ve charged slides, EGFR Monoclonal Mouse antibody (Anti-Human Primary antibody) Abcam, (Cambridge, UK for detection EGFR proteins not specific. Three scoring scales were connected at 40X goal as pursues Zero score with no stain, +1 score, faint staining of cytoplasmic considered (weak). +2 score, moderate staining of smooth cytoplasmic considered (moderate). +3 score, intense staining of granular cytoplasmic tumor cells considered.

C. **Molecular study : DNA Extraction** was prepared from Paraffin embedded Lungcancer specimens with sections thick 5-10 μm. Isolation of DNA genomic protocol according to Genomic DNA Mini Kit (Geneaid biotech. Ltd., Feline. No GS100,), Lot no. FE16205-N), and The extracted DNA concentration (ng/μL) assessed by using Nanodrop Spectro-photometer according to.

Amplification of the gene by the reaction prepared it used 8 μl of PreMix and 2μl of DNA samples, 1μl of primers (0.5μl forward 5'-ATCCAGAAGGTGAGAAAGATACATTCTC-3' and 0.5 μl reverse from 5'-CCTGAGGTTGAGGCATGGA-3') and added 8.5 μl distilled water and 0.5MgCl2 mixed with vortex. DNA was amplified Initial Denaturation 5 min in 94°C for 35 cycles at 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, extension at 72°C for 5 minutes, and Performing PCR of samples. The 1st PCR products done using MseI 10 IU at 37°C over-night PCR aliquots were used as a template for the 2nd round of PCR amplification as the first round PCR conditions but for 40 cycles. The PCR products of the 2nd amplification was analyzed on 12% Page Via Silver staining.

**Statistical analysis:** Statistical Package for the Social Sciences (SPSS) Version 23, were expressed in the of mean & standard deviation form (mean±SD), Pearson t-test and Chi-square test, correlation with (P < 0.05).

**Results**

I. **Clinicopathologic Characteristic in Patients and Control:** Our results have , the patients Male33, Female17 while control Male19, Female11, confirmed the mean age of patients group was 51.84 +/- 15.70 years and the median age of patients was 54 years (range 13-76 years) while the mean age of control 39.266 +/- 21.43 and median age of control was 38.500 years (range 10-79) years, The Male/female ratio of patients were 2:1 while the control were 1.75:1. Male/ female ratio there both of patients and control was no statistical difference (P> 0.05), The distribution of patients according to site of tumor were as the following (29) patients had a right sided lung tumor, accounting for 58 %, while (21) patients had a left sided lung tumor, accounting for 42 %. show in (Table 1 and Figure 1), our study by using Kendall’s tau-b yielded not correlated between stage and grade.
Table 1: Clinicopathologic Characteristic in Patients and control

<table>
<thead>
<tr>
<th>Variable</th>
<th>No%</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age( years) patients</td>
<td>was51.84</td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>+/-15.70</td>
<td>0.809</td>
</tr>
<tr>
<td>Gender Male</td>
<td>39.266</td>
<td>0.507</td>
</tr>
<tr>
<td>Female</td>
<td>+/-21.43 to  33(66%)</td>
<td>0.261</td>
</tr>
<tr>
<td>site of tumor right sided</td>
<td>17(43%)</td>
<td></td>
</tr>
<tr>
<td>left sided</td>
<td>29(58%)</td>
<td></td>
</tr>
<tr>
<td>grade of tumor</td>
<td>21(42%)</td>
<td>0.1</td>
</tr>
<tr>
<td>well differentiated</td>
<td>25(50%)</td>
<td>0.4</td>
</tr>
<tr>
<td>Moderately differentiated</td>
<td>16(32%)</td>
<td></td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>9(18%)</td>
<td></td>
</tr>
<tr>
<td>stage of tumor stage I</td>
<td>16(32%)</td>
<td></td>
</tr>
<tr>
<td>stage II</td>
<td>26(52%)</td>
<td></td>
</tr>
<tr>
<td>stage III</td>
<td>8(16%)</td>
<td></td>
</tr>
</tbody>
</table>

II-Immunohistochemical Expression of EGFR:

<table>
<thead>
<tr>
<th>Variables</th>
<th>Negative</th>
<th>Positive</th>
<th>Total</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>25 (75.8%)</td>
<td>8 (24.2%)</td>
<td>36(72%)</td>
<td>0.410</td>
</tr>
<tr>
<td>Female</td>
<td>11 (64.7%)</td>
<td>6 (35.3%)</td>
<td>14(28%)</td>
<td></td>
</tr>
<tr>
<td>Sit</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>19(90.5%)</td>
<td>2 (9.5%)</td>
<td>21</td>
<td>0.01</td>
</tr>
<tr>
<td>Right</td>
<td>17(58.6%)</td>
<td>12(41.4%)</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Stage I</td>
<td>9(56.25%)</td>
<td>7(43.75%)</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Stage II</td>
<td>13(50%)</td>
<td>13(50%)</td>
<td>26</td>
<td>0.54</td>
</tr>
<tr>
<td>III</td>
<td>6(75%)</td>
<td>2(25%)</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Grade I</td>
<td>5 (83.3%)</td>
<td>1 (16.7%)</td>
<td>6</td>
<td>0.416</td>
</tr>
<tr>
<td>II</td>
<td>11(61.1%)</td>
<td>7(38.9%)</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>20 (76.9%)</td>
<td>6 (23.1%)</td>
<td>26</td>
<td></td>
</tr>
</tbody>
</table>

In our study showed that highly correlated difference in EGFR Positive between patients and control subjects,. Positive EGFR was limited to patients with lung carcinoma (28%) whereas non of control a positive EGFR expression (0%). EGFR of IHC expression was detected as brown cytoplasm. The score of EGFR was as follows: score 1 accounted for 3 (21.42%) of cases, score 2 was seen in 9 (64.28%) of cases and score 3 was present in 2 (14.28%) of
Table 2 Immunohistochemica of EGFR in Patients

cases show in figure(5,6,7,8) , No correlated were found both gender and EGFR expression patients with Lung carcinoma (P> 0.05), No statistically significant difference was observed for between Grade and Stag with EGFR expression but significant with Sit of Tumor (P> 0.05) show the( Table2 & figure2 ).

III- Molecular: The associated of gene EGFR exon19:

Gene EGFR deletion of exon19 were analyzed using mutant-enriched polymerase chain reaction (PCR) was not detected in all patients and control as shown in figure(3).

Discussion

In the present study the mean age of patients with carcinoma was 51.8 4+/− 15.70 years and the lung median was 52 years while the age range was from 14 years through 79 years, expressed that smoking is the main source of lung disease and the explanations behind the predominance of smoking in Iraq are people conduct and the social and social condition that advance smoking and it was accounted for that 85-90% of lung malignant growth patients Torre et al17 expressed that older age was related with disease advancement because of biologic components that incorporate DNA damage over time and shortening telomeres, Al khuzaie et al18 stated a study the first most common site in males on newly diagnosed Iraqi cancer patients. performed during a 3 years period (2005-2007),Al-Hashimi and Wang19 the reported among 2000 and 2010 demonstrated the rate is most noteworthy (19%) in the age gathering 60–69 years in Iraq/Mousl, Jaloudi et al20, An aggregate of 25 NSCLC patients were enrolled in the examination at standard (7/25 Bahrain; 11/25 UAE; 7/25Qatar) illness, The mean age of the was 56.9 which are slightly higher than that of the present study In a recent study done in Iran, on 1353 patients with Lung carcinoma, the mean age was 60 years and the age range was16-94years Khosravi et al22 these findings are substantially higher than that of the present study. It’s shown that changes in prevalence and types of tobacco smoking , physical activity, air pollution in indoor , radon environments can affect the pattern of lung cancer in terms of gender, While the men age range was (56 years) in India22. In an Saudi Arabia, Kuwait and UAE the men age range were (66 years) 23, the multiple studies which are higher than
of the present study. Male to female ratio was 2.4:1 in the present study one of the hypotheses is that results further suggest that smoking, DNA capacity repair, alcohol consumption and obesity another hypotheses hormonal factors such as estrogens which is not present in normal lung tissue Temraz et al. reported a male to female ratio of Lebanon 2.5:1, which is again, in accordance with the present study while Jaloudi et al. a total of 25 NSCLC patients (Bahrain; UAE; Qatar) disease were 2.8:1 which is again, in accordance with the present The present study showed that 54% of tumor masses were located in the Right region and that 46% of cases were located in the Left region. These findings differences in patients characteristics, According to Jaloudi et al. the tumor masses of lung is the most common in the upper right lobe approximately (57.1%) of the cases and followed by the upper left lobe. The present study showed that the majority carcinoma (50%) had a well differentiated grade I histological pattern, its findings differences in patients characteristics, other factors that showed a prognostic effect independent of disease grad include age and gender several genetic bio markers, while other factors like obesity and smoking history was associated with lung cancer. Well to Moderately differentiated morphology was the major histological grade reported by some authors. The present study showed that majority of patients enrolled in the present study (52%) had stage II disease. The finding of the present study that majority of patients had stage II disease is in agreement with many authors.

**Conclusion**

Our results indicate the non correlation between stage & grade of disease with the age of patients and Expression of EGFR of Immunohistochemical method highly significant difference between patients and control healthy, had found as not significant with age, gender, grade, stage, but significant with sit while EGFR significant difference between patients and healthy controls but not significant with, gender, sit of tumor, grade and stage the end non correlation between Expression of EGFR Immunohistochemical and mutation.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Department of Biology, Faculty of Science, University of Kufa/Iraq and all experiments were carried out in accordance with approved guidelines.

**References**


Depression in Relation to Serum Ferritin a Cross-sectional Study at Najaf Governorate

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Psychiatrist at the Department of Medicine, Medical College, University of Kufa, Head of Department of Psychiatry Al-Hakeem General Hospital

Abstract
depression is a common psychiatric disorder that leads to disability and increases morbidity and mortality, and iron deficiency is a common nutritional disorder in general population that lead to change in brain function and affecting the quality of life. Methodology; a cross-sectional study that was conducted at Iraq Al-Najaf governorate, patient with depression was (51), and control group was (95), both were assessed by Beck depressive inventory Arabic version for the scoring of depressive symptoms, for both group serum ferritin was evaluated from fasting blood sample. Result; The age of participants (mean=36.25 years), the male was (55) while the female (91), of them (88) were married, there is significant differences between groups in age group (36-45) years and female gender while not to another variable. serum ferritin was significantly deferent with P-value (0.001). Conclusion; there is a strong relationship between the level of serum ferritin and depression with inverse correlation.

Keywords: depression, serum ferritin, Beck depressive inventory, Najaf

Introduction
Depression is a common psychiatric illness, that leads to disabling and lowering of productivity, and worsening the outcome of chronic illnesses due to nonadherence and increases the cost spend on these illnesses. It is regarded as one of the leading cause of death. Symptoms of depression also had a high prevalence, and it has an intimate relationship with quality of life, social interaction, attempts of suicide and physical health. Depression rate was 8 to 20% in the United States, and its causes either inherited (genetic) and cannot be changed, or acquired can be changed or modified.

There is a firm relation between development of depressive symptoms and nutrition, role of vitamins deficiency (like vitamin C, niacin, Vitamin B12, and folic acid) in mood disorder had been confirmed, iron level play a significant role in brain function, person’s behavior, and also had effect on depression (in both negative and positive ways).

Iron deficiency is a common nutritional problem, concerning socio-economic state in both developed and developing countries, its consequences lead to many mental, emotional and behavioral change and lead to mood disturbance. Ferritin is an intracellular protein that plays a role in store and releases iron and most widely biomarker that used to determine the iron level, and it is important to notice that inflammation alter the level of serum ferritin. A national survey found that iron deficiency in women was 11% (mostly premenopausal) and in men 4%; without developing anemia.

There is a lot of questionnaires, scales or inventories that used to assess depression but Beck depressive inventory was still the most widely used and more valid and specific in detection of depression.

Methodology: One hundred and forty-six persons were enrolled in this cross-sectional study that conducted at Iraq Al-Najaf governorate from January through December 2018.

Inclusion criteria; any participant that age 18 years or more, participants that had a will to be involved in this study.

Exclusion criteria; patients that had inflammatory process (with high ESR level) where ferritin is released.
in response to inflammation (acute phase)\(^1\), those how had anemia (any person had the level of Hgb of less than 12g/dl), participant that take medication changing the mood, patients that had depression secondary to medical condition, participants that take corticosteroid\(^2\), patient with psychotic symptoms (lack of insight), those with co-morbid psychiatric disorder, those who receive multivitamin or iron, those how had chronic debilitating illness, and those who had difficulty in communication or agitated.

All selected participants are provided a written consent to be part of this study.

The patients group where selected from Psychiatric department\(1\) Al-Hakeem general hospital outpatient clinic and private psychiatric clinic, and the control group was selected from community setting and from relatives of patients that consult other outpatient clinics (other than psychiatric clinic), the study group was (\(n=146\)), the patient with depression group (51, 34.9%) and the control group (95, 65.1%), the age of the participants ranging from 18 to 56 years.

At the beginning, the participant’s data was taken and a question about medical history, history of medications use such as (\(B\)-blocker, corticosteroid, calcium channel blocker, iron or vitamins supplement, anticonvulsant) are obtained.

All participants that enrolled in this study (both groups) were assessed by using the Beck depressive inventory (BDI) Arabic version\(^2\), to evaluate the presence of depression and for scoring, although the case group was assessed also by ICD-10 criteria\(^3\) of depression to confirm the diagnosis. Scoring of BDI was from zero to 63 point in 21 items each had 4 questions scoring (0, 1, 2, and 3), more than 10 regarded as having depression. The BDI may be given to the patient for self-administration or group administration, but it should be verified that the patient understands the purpose and the answering method for the test, for those how cannot read; the researcher read to them to overcome this obstacle. The control group was assessed by the general health questionnaire (GHQ-30)\(^4\) to exclude the presence of any psychiatric or mental illnesses (by an expert psychiatrist).

A blood sample was taken from both groups (those with depression and those free from mental illnesses) after fasting for at least 8 hours before taking the blood sample, then assessment of serum ferritin level was done immediately after taking the sample by using (ELISA), assessment of hemoglobin level (Hgb); to assess presence or absence of anemia, and also evaluation of ESR to exclude inflammatory process; normal range of ESR is (for the age less than 50 years 0-15mm/hr for male and 0-20mm/hr for female, for the age more than 50 years 0-20mm/hr for male and 0-30mm/hr for female)\(^5\), normal value of serum ferritin ranging 0.015-0.2mg/l and level below that regarded as iron deficiency. While the level of Hgb of lower than 12g/dl regarded as having anemia\(^6\).

Data were entered and analyzed using the statistical package for social sciences (SPSS) version 24, 2015, IBM, USA. Descriptive statistics were presented as mean, standard deviation, and percentages. Student’s t-test (independent model) was used to compare the mean of the serum ferritin in comparison of patients and control groups. Logistic regression was used to determine the relationship in both groups. and less than0.05 of \(P\)-value was regarded as significant.

**Results**

The age of participants was (mean=36.25 years ± 4.1year), it is predominant at 36-45 years, the female was dominating both groups, being married was more than being single (divorced widowed or never married), while educational state the secondary school was more in patients group and primary school was more in control group. Most of the participants had work (98, 67.12%) and it is not a significant \(P\) value (0.66). The age period (36-45) years and female show the significant difference with \(P\) value (0.001 for the case group and 0.01 for control group), while it is not significant for other variables more than 0.05, as shown in table (1).
### Table 1. The demographic characteristics of the sample.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Case group (Patients with depression)</th>
<th>Control group</th>
<th>p. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥25</td>
<td>8</td>
<td>15.67</td>
<td>16</td>
</tr>
<tr>
<td>26-35</td>
<td>13</td>
<td>25.49</td>
<td>24</td>
</tr>
<tr>
<td>36-45</td>
<td>20</td>
<td>39.21</td>
<td>35</td>
</tr>
<tr>
<td>&gt;46</td>
<td>10</td>
<td>19.6</td>
<td>20</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>16</td>
<td>31.37</td>
<td>39</td>
</tr>
<tr>
<td>Female</td>
<td>35</td>
<td>68.62</td>
<td>56</td>
</tr>
<tr>
<td>Marital state</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Being single</td>
<td>22</td>
<td>43.13</td>
<td>36</td>
</tr>
<tr>
<td>Married</td>
<td>29</td>
<td>56.86</td>
<td>59</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not read or write</td>
<td>3</td>
<td>5.88</td>
<td>10</td>
</tr>
<tr>
<td>Read and write</td>
<td>7</td>
<td>13.72</td>
<td>15</td>
</tr>
<tr>
<td>Primary school</td>
<td>13</td>
<td>25.49</td>
<td>31</td>
</tr>
<tr>
<td>Secondary school</td>
<td>18</td>
<td>35.29</td>
<td>23</td>
</tr>
<tr>
<td>Institution</td>
<td>9</td>
<td>17.64</td>
<td>11</td>
</tr>
<tr>
<td>Higher education</td>
<td>1</td>
<td>1.96</td>
<td>5</td>
</tr>
<tr>
<td>Work</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Have no work</td>
<td>19</td>
<td>37.25</td>
<td>29</td>
</tr>
<tr>
<td>Have work</td>
<td>32</td>
<td>62.74</td>
<td>66</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>100</td>
<td>95</td>
</tr>
</tbody>
</table>

The serum ferritin ranging from 1 and 129 µg/l for case group and in control group ranging from 2 and 193 µg/l, and the student’s t-test clarify the difference between the study groups was significant ($P=0.001$). The correlation coefficient in the relation of S. ferritin and depression was (- 0.158) and it is significant ($P<0.05$), and this indicate a negative correlation. Assessment the odds ratio; the ferritin level was changed into phantom variable (level equal or less than 15 and more than 15), the relation to depression was assessed by using logistic regression and showed that the change from normal toward low S. ferritin level increase the depression odd’s by (1.93) and ($P<0.05$), as shown in table (2).
Table 2. Differences between the study group’s beck score, S. ferritin, Hgb and ESR (mean and standard deviation)

<table>
<thead>
<tr>
<th></th>
<th>Case group (Patients with depression)</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beck score (M± s.d.)</td>
<td>17.1 ± 4.5</td>
<td>4.2 ± 1.7</td>
</tr>
<tr>
<td>S. ferritin (µg/l) (M± s.d.)</td>
<td>21.85 ± 11.3</td>
<td>39.45 ± 18.2</td>
</tr>
<tr>
<td>Hgb (g/dl) (M± s.d.)</td>
<td>12.8 ± 0.5</td>
<td>13.6 ± 1.2</td>
</tr>
<tr>
<td>ESR (M± s.d.)</td>
<td>8.7 ± 2.1</td>
<td>9.1 ± 2.3</td>
</tr>
</tbody>
</table>

**Discussion**

In this work, we examine the relation of depression and effect of S. ferritin level, and shows that the level of ferritin (mean) was near normal or normal in both groups with deference about 17µg/l lower in the case group, and that goes with finding of (Vahdat M. et.al 2007) 26. And it dislike study of (Hunt and Peland, 1999) in the United States where they found no relationship between depression and level of ferritin, Hgb or iron levels 27, and that may due to in there work they did not exclude participant that take iron supplement or taking medication that affects level of ferritin or having chronic illnesses or had inflammatory diseases. Although, all these may increase ferritin level 21. other studies also found no significant relationship between depression and level of ferritin 27-30. lowering the s. ferritin level association with depressive indicated the possibility that the iron plays a role not only in oxygenation of brain cells but also in enhancing brain function and the provoking of depressive symptoms 19, 26, 31-34. Iron deficiency results in impairment of monoamine metabolism and also affect brain myelination, which result not only defects motor skills, memory, and learning capacity, but also produce psychological and emotional disturbance 15, 16. Iron play role in the synthesis of Dopamine (which a neurotransmitter that had an important action in mood regulation, lowering of dopamine level produce depression), also a study by (Kaplan et al. 2003) found that the level of blood dopamine was low in patients with depression 8.

At the same time, studies show that elevated s. ferritin level in patients may associated with development of depressive symptoms which is not examined in this study. A study by (Baune BT et.al 2010) shows that normal or increased ferritin levels may have a role in the development of depressive symptoms, as a part of the inflammatory process 19. Other suggestion was that whether serum changes in iron level did not reflect its uptake in the brain at all 28. Also, data from the experimental study suggest that brain iron uptake may not depend on plasma transferrin, transferrin saturation, or regional brain iron.

Another work done in the United States by (Lozell et al. 2000) study infant with IDA and after 10 years follow up they found that those children are more liable to get depression, anxiety, and concentration problem than other without IDA.

The study shows an inverse correlation between the level of ferritin and depression as the correlation coefficient (-0.158) and (P<0.05), goes with the finding of (Vahdat M. et al. 2007) 26, and this may reflect the role of low iron in developing depressive symptom.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Psychiatrist at the department of medicine, Medical College, University of Kufa, Iraq and all experiments were carried out in accordance with approved guidelines.

**References**

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Histological Evaluation the effectiveness of Aloe Vera oral gel Application on Gingiva Subjected to Dental Light –emitting Diodes

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Abstract

Background: Aloe Vera (AV) is a medical plant used in dentistry with multiple uses proved as antiseptic, antifungal, antiviral and anti-inflammatory and very good in building the immune system with a natural compound that has no side effect, for that reason we use it as protective layer from the heat that generated from LED light cure during the filling or cementation procedure or others.

Materials and method: Thirty-five male New Zealand rabbits of weight 1.5 –2kg were used in this study; they were divided into three groups. First group (5 rabbits) the gingiva of upper incisors is not exposed to dental LED light cure (control group), Second group (15 rabbits) which the cervical gingiva of upper incisors which is separated from light source by double transparent celluloid strips, cervical gingiva is exposed to LED curing light 6 seconds three times with 20 seconds interval. Third group (15 rabbits) same as 2nd group but gingiva covered by Aloe Vera oral gel. Animals’ scarifications were done at (0 day) immediately after exposure to curing light ,3 days,7 days healing intervals. Routine processing and sectioning technique was performed for histological evaluation.

Results: Histological findings indicated variation in epithelial thickness, irregularity in basal cells layer and number of blood vessels of 2nd & 3rd experimental groups from 1st control group. The highest mean values of all measured Histomorphometrical parameters were observed in 2nd group 3 days after dental LED light cure exposure.

Conclusion: Aloe Vera oral gel may be used on gingiva as a protective layer to decrease side effects of LED light cure on gingiva.

Key words: Aloe Vera, gingiva, Light –emitting Diodes, rabbit

Introduction

Aloe vera(AV) is a medical plant used in dentistry and proved as antiseptic, antifungal, antiviral and anti-inflammatory properties \textsuperscript{1}. The magical healing property of Aloe Vera is due to a compound called glucomannans, Glucomannans enhances fibroblast growth factor and encourages the proliferation and activity of these cells result in more number of collagen and elastic fibers. Low molecular weight compounds present in aloe inhibit the production of reactive oxygen free radicals from activated human neutrophils \textsuperscript{2}Because of the effect of LED light cure with high intensity of 1500-2000mw\watt, and the heat generated during the procedure of filing or cementation. This high intensity can damage nearby tissue like oral mucosa Although this temperature range depends on LED type, radiant emittance, and tooth characteristics,\textsuperscript{3} there is a consensus that the use of some LED because of the high temperature generated to approximately 41°C. Although the temperature increase that cause severe thermal damage on the gingival tissue is still unknown, approximately 67 % and 77 % of the tissues exposed to light for 40 and 60 seconds,
respectively, developed a gingival lesion. For that reason AV was used in this study as a protective layer on the gingiva because of anti-oxidant effect also, and it act as moisturizer, soothing and cooling agent.

**Materials and Method**

The materials used in the present study were dental diode curing light 1800 mW/cm² (China), Aloe vera oral gel (Drwolfe, New México), transparent celluloid strips (PD,SWESS) anesthetic solution: Ketamine hydrochloride 50 mg and Xylazine 2%, formalin 10%, ethanol alcohol 96%, xylol, paraffin wax, and Hematoxylin and Eosin (H&E) stain. Thirty-five male New Zealand rabbits of weight 1.5 –2kg were used in this study, they were divided into three groups. First group (5 rabbits) the gingiva of upper incisors is not exposed to dental LED light cure (control group), Second group (15 rabbits) which the cervical gingiva of upper incisors which is separated from light source by double transparent celluloid strips. cervical gingiva is exposed to LED curing light 6 seconds three times with 20 seconds interval. Third group (15 rabbits) same as 2nd group but gingiva covered by Aloe Vera oral gel. Animals were scarified by an overdose of anesthetic solution at (immediately after exposure to curing light (0 day), 3 days, 7 days) intervals. The specimens were fixed in 10% buffered formalin for 24h, then gingival tissue dehydrated with alcohol and embedded in paraffin. Sections of 5μm were prepared in the usual fashion, and stained with hematoxylin and Eosin stain.

Histological evaluation was performed using light microscope (OpticaB-350,Italy). Histomorphomatrical parameters are measure the epithelial thickness (EP, TH.) , percentage of basement membrane length to epithelial surface length (BML\EPSL) and number of blood vessels (B.V). Measurements were performed by image processing software program (ImageJ.exe). Microphotographs were taken by a camera (AIPTEK HD 1080P, China) attached to the microscope at power X40.

**Statistical Analysis**

The data were entered and analyzed using SPSS 18.0. Mean ± SD Min. and Max. were given for quantitative variables. Tukey HSD test was applied to observe associations between groups and morphological changes. A P-value ≤0.05 was considered as statistically significant.

**Results**

**Histological evaluation:**

**Fig. 1: Hematoxylin Eosin staining of the gingival tissue of rabbits in 0 day. (A) control**
Histomorphometrical analysis

The highest mean values of all measured parameters were observed in 2nd group 3 days after dental LED light cure exposure (Table 1). While at 3 days healing interval: A high significant difference noticed in this healing period among all groups in all parameter, Highest mean value of all measured parameters recorded in 2nd group((EP.TH.) 0.586, Percentage of basement membrane epithelial surfaces 361.8 and blood vessels number was 38.8. At 7 days, 3rd experimental group shows a normalcy gingival epithelium features when there are a non significant differences in(EP.TH.) and blood vessels number between 1st and 3rd group, But in 2nd experimental group still there are high significant differences from other groups.

Fig. 2: **Hematoxylin Eosin** staining of the gingival tissue of rabbits in the 3 days interval (A) 2nd group show irregular both epithelium surface and basement membrane (B.M)

Fig. 3: **Hematoxylin Eosin** staining of the gingival tissue of rabbits in the 7 day healing
Table 1: Descriptive statistics and group differences in 0 day duration of measured epithelial parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>N</th>
<th>Mean</th>
<th>S.D.</th>
<th>Min.</th>
<th>Max.</th>
<th>F-test</th>
<th>p-value</th>
<th>Comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial thickness</td>
<td>I 5</td>
<td>0.24</td>
<td>0.016</td>
<td>0.22</td>
<td>0.26</td>
<td>6.328</td>
<td>0.013</td>
<td>I-II</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>II 5</td>
<td>0.29</td>
<td>0.025</td>
<td>0.26</td>
<td>0.32</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>III 5</td>
<td>0.286</td>
<td>0.030</td>
<td>0.25</td>
<td>0.32</td>
<td></td>
<td></td>
<td></td>
<td>I-III</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>II-III</td>
</tr>
<tr>
<td>Percentage of basement membrane/epithelial surfaces</td>
<td>I 5</td>
<td>130.6</td>
<td>6.107</td>
<td>125</td>
<td>139</td>
<td>87.674</td>
<td>0.000</td>
<td></td>
<td>I-II</td>
</tr>
<tr>
<td></td>
<td>II 5</td>
<td>167</td>
<td>2.739</td>
<td>164</td>
<td>171</td>
<td></td>
<td></td>
<td></td>
<td>I-III</td>
</tr>
<tr>
<td></td>
<td>III 5</td>
<td>151.2</td>
<td>3.493</td>
<td>147</td>
<td>156</td>
<td></td>
<td></td>
<td></td>
<td>II-III</td>
</tr>
<tr>
<td>Numbers of blood vessels</td>
<td>I 5</td>
<td>20.2</td>
<td>1.924</td>
<td>18</td>
<td>23</td>
<td></td>
<td></td>
<td>0.361</td>
<td>0.704</td>
</tr>
<tr>
<td></td>
<td>II 5</td>
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<td>2.550</td>
<td>18</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>III 5</td>
<td>21.4</td>
<td>2.302</td>
<td>18</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Visible Light Cure (VLC), a blue light with a wavelength between 400-500 nm. It can cause thermal reaction (5). Complaints from patients in connection with light-curing procedures have been reported, including experience of Burning sensations in teeth and in oral tissue. We used rabbits of New Zealand breed, as morphological features of rabbits are closely similar to human gingival mucosa as well as ease of their availability (Nanci, 2008). This study showed histological changes induced by LED dental light cure (1800mW/cm²) can incite thermal energy in biological tissues which can cause burns. Visible light cure absorbed by Cytochrome form excessive Reactive Oxygen Species (ROS) which can cause cell damage (Voskanyan KS, 2009).

Increase in (EP.TH.), (B.V) and (BML/EPSL) in response to elicit tissue damage this agree with Abdul khaliq in 2016 observed Acanthosis and the hyperplasia of prickle cell layer. In present study increase in blood vessels in 3 days interval can attributed to increase blood supply to overcome oxidative stress. Regarding 3rd group, Using Aloe Vera gel protection shows non significant difference between 1st and 3rd groups in (EP.TH.) and (B.V) which resemble normal gingiva this may be due to anti-inflammatory effect and angiogenesis of Aloe Vera gel.

Conclusion

Aloe vera oral gel may be used on gingiva as a protective layer to decrease side effects of LED light cure on gingiva by decrease (EP.TH.), (B.V) and (BML/EPSL) changes and accelerates return to normal condition.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the AL-Rafidain University Collage, Baghdad-Iraq and all experiments were carried out in accordance with approved guidelines.

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A Concordance Study between Polymerase Chain Reaction Assay and Conventional Culture-based Methods for Detection of *Fusobacterium necrophorum*

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**Abstract**

Foot rot due to *Fusobacterium necrophorum* is an important clinical economic disease in ovine, in order to inform identification and antibiotics selection, Conventional culture-based method and Polymerase chain reaction assays had been proposed to provide a good inter-rater reliability between these techniques, this study was aimed to differentiate the accuracy of conventional culture-based method and PCR technique for identification of *Fusobacterium necrophorum* in an ovine foot rot.

**Method:** The current study was conducted on three farms of Kerbala governorate (AL-husseinyia, Al-Hurr and Ain Al-tmor cities) during the period from October 2017 to April 2018, pathological swab samples of the interdigital region of affected (n= 40) sheep and (n=40) goat were enrolled in this study, after the collection of swabs, the samples were divided into two partitions, each swab of the first partition was immediately collected into lighten tube containing 5 mL of thioglycollate broth enriched with 0.5% ground sheep and was transmitted to the laboratory for conventional culture-based method and the another partition of swabs was sealed with other cooled tubes containing 5 mL of thioglycollate broth enriched with 0.5% ground sheep for the laboratory of molecular investigations.

**Results:** *Fusobacterium necrophorum* was identified in the pathological samples by the two following depended assays: and (46) swabs were positive by conventional culture-based method and (44) swabs were positive with PCR assay, Cohen’s kappa coefficient revealed P value for conventional culture-based methods vs. PCR assay of 0.056 and the current study was demonstrated a highly identification rates of *Fusobacterium necrophorum* in conventional culture-based methods rather than PCR assay.

**Conclusions:** Current study documented conventional culture-based method and PCR assays were facilitate the identification of *Fusobacterium necrophorum* in case of presumed ovine foot rot, it also concluded that molecular assays should be depended in confirmatory detection of *F. necrophorum* in affected animals.

**Key words:** Foot Rot, *Fusobacterium necrophorum*, PCR, Sheep, goat

**Introduction**

Foot rot is a highly contagious disease of ungulates characterized by exudative inflammation with offensive odor, resulting in acute or sub-acute inflammation and necrosis of the tissues of interdigital space of sheep. It is worldwide distribution but highly incidence rates in cold and wet regions, it may reach to peak occurrence in hot and wet summer in both sheep and cattle.

¹³ reported that foot rot is a frequent cause of lameness in sheep with severe economic losses in herds because of decreased weight gain and treatment costs. Also, lame bulls will not breed and occasionally, animals with severe and complicated disease may need to be discarded from the herd. *Fusobacterium necrophorum* is a gram-negative, pleomorphic rod-shaped and anaerobic bacterium, was associated with many necrotic lesions in animals and humans, it is a normal inhabitant of the gastrointestinal tract in animals and humans and this
pathogen is existed within the ruminal fluid of ruminants at concentrations of \(10^5\) to \(10^6\) per gram with variations occurring depended upon the diet of the animal \(^{30}\).

Kumar \(^{14}\) found that the *Fusobacterium necrophorum* in sheep was the primary etiological agent of liver abscesses, and two subspecies; subsp. *necrophorum* and subsp. *funduliforme*, were different morphologically, biochemically, and by the molecular analysis. The subsp. *Necrophorum* is the more virulent and occurs more frequently in liver abscesses than the subsp. *funduliforme*.

Moreover, \(^{16}\) recorded many toxins in *F. necrophorum*, such as leukotoxin, endotoxin, hemolysin, hemagglutinin, proteases, and adhesin., have been considered as virulence factors. Leukotoxin is a major virulence factor, it is a secreted protein of high molecular weight, active specifically against leukocytes of ruminants and the complete sequence of nucleotides of the leukotoxin operon of *F. necrophorum* has been detected., the operon consists of three genes (*lktBAC*) of which the second gene (*lktA*) is the leukotoxin structural gene.

Previous study was utilized the *lktA* gene of *F. necrophorum* to determine *F. necrophorum* variants in cattle, sheep and goats which diagnosed with foot rot., It was demonstrated that a particular variant of *F. necrophorum* *lktA* (designated variant A) tended to be determined in sheep with foot rot and this *lktA* sequence matched the strain of *Fnn* (NCTC 10575) which isolated from bovine with liver abscess \(^{34}\).

**Materials and Method**

**Animals:** eighty ovine (40) sheep and (40) goats were conducted in the present study from un-organized three farms in kerbala city which included (AL-husseinyia, Al-Hurr and Ain Al-tmor cities), all data like age, gender of animals and duration of the study were recorded., the study was extended from October 2017 to April 2018. At all (80) animals were observed on the following properties, the site of lesions of foot rot (unilateral or bilateral) and the claws were cleaned and better trimmed for investigating of bacterial lesions and achieving the pathological swab samples.

**Collection of Samples and Bacterial Cultivation**

Direct swab smears were collected from the interdigital lesions of the affected animals and directly stained with grams stain. After collection of swab samples, they were divided into two partitions in lighten tubes containing 5 mL of thioglycollate broth enriched with 0.5% ground sheep hoof (Aguiar), the first partition was transmitted anaerobically to the medical microbiology laboratory for bacterial cultivation, then, the swab samples were incubated anaerobically at 6% CO2 and 37°C for 24 hours, and cultivated on Colombia blood agar plates, incubated anaerobically at 37°C for 48 hours, then, the cultivated plates were examined morphologically to determine the characteristic colonies of *Fusobacterium necroforum* which appeared on the culture media and biochemical characterization of the colonies of *F. necrophorum* according to the procedures of \(^{30,80}\) were performed.

**Biochemical characterization**

The following biochemical activities included Catalase test, Oxidase test, Indole test, MR/ VP test, H2S production, Litmus milk, DNase test, Lipase test in addition to fermentation of the following sugars-Glucose, Maltose, Fructose Sucrose, Galactose, Lactose, Mannitol and Mannose as well as Motility test were performed.

**Molecular identification:**

The second partition of swab samples were used for PCR assay for molecular detection of *Fusobacterium necroforum lktA* gene according to the following procedures.

**DNA Extraction:**

Preparation of DNA :- each of the contains of 5 ml of enriched broth of swab samples were centrifuged and washed with sterile normal saline and suspended in 2 ml of 50 mM Tris-HCl buffer (pH 8.0) containing 5 mM EDTA and 50 mM NaCl. and the suspension which prepared was incubated with shaker incubator at 37°C for 30 min and used for DNA extraction according to the instructions of the commercial kit (the Presto™ Mini gDNA Bacteria Kit/ Korea) and amplification of *Fusobacterium necrophorum lktA* gene was performed according to the following program.

**Primer and PCR polymerization:**

PCR technique was performed in a final reaction volume of 25 µl master mix in a thermal cycler (Biometra – Germany) with 5 µl of extracted DNA and 1 µl of each of specific primers for *leukotoxin A* gene of
**F. necrophorum** sub sp. *Necrophorum* (lktA) (Forward 5-ACAATCGGAGTAGTAGGTTC-3) and (Reverse 5-ATTTGGTAACTGCCACTGC-3) 403 bp, they were designated by [5] based on the published *F. necrophorum* sub sp. *necrophorum* leukotoxin gene sequence (GenBank accession number DQ672338). PCR products were electrophoresed in 1.5% agarose gel containing ethidium bromide (5μl /100ml) with DNA ladder at along with 100 bp. and PCR products were visualized as a single orange fluorescent band under UV light.

**Statistical Analyses**

Chi square and Cohens Kappa coefficient tests were used to investigate the degree of concordance between the two identification assays (conventional culture and PCR), A P value <0.05 was considered as statistically significant by using statistical analysis of social science data software version 22.

**Results and Discussion**

Obtained results were depended cohens kappa coefficient (K) to measure inter-rater agreement between the two identification assays (conventional culture and PCR), they found the moderate interobserver agreement (0.645) between the two assays on comparing with the results of routine diagnosis have cohens kappa coefficient 0.645, with no significant association between the two identification assays (P value >0.05), according to the kappa interpretation there was a relatively interobserver agreement found table 1.

<table>
<thead>
<tr>
<th>Inter-rater agreement</th>
<th>Conventional culture-based methods</th>
<th>PCR</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ve</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-ve</td>
<td>28 (82.4%)</td>
<td>8 (17.4%)</td>
</tr>
<tr>
<td></td>
<td>+ve</td>
<td>6 (17.6%)</td>
<td>38 (82.6%)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>34 (34.5%)</td>
<td>46 (65.5%)</td>
</tr>
</tbody>
</table>

Previous studies revealed that conventional culture-based methods was more robust measurement for detection of *F. necrophorum* than PCR assay, interestingly, the recovery of *F. necrophorum* by culture-based methods was similar to that of PCR and they were consistent with the results of another study demonstrating that PCR was predominantly and useful technique for detecting of low concentrations of *F. necrophorum* [2]. In spite of these different clinical findings, there was a considerable confusion about the causative agents, particularly related to duplication of names for *F. necrophorum* but also due to misattribution of a role of other etiological organism such as *Streptococci* [1], or inadequate anaerobic culture methods. Thus, increasing the likelihood of occurrence false positive.

The clinical examination of (80) infected sheep with foot rot in the current study have been revealed the development of fever in affected animals, the temperature ranged between 40-41°C according to severity of foot rot lesions, the increase of body temperature reached (41°C) in (45) animals with marked increase in heart beats to (90 b/m) and respiratory rate to (45 b/m) as demonstrated in another similar study [21]. During the period extended from October 2017 to April 2018 the examination of (80) sheep suffered from foot rot lesions in both gender with different ages in Karbala governorate revealed various degrees of lameness in one or two limbs. These clinical signs were agreed with the study of [6] and [26]. The typical lesion occurs in the skin at the top of the interdigital cleft and takes the form of a fissure with the swollen and protruding edges might extended along the length of the cleft or be confined to the anterior part or
the part between the heel bulbs. Exudates secretions were few but the edges of the fissure were covered with necrotic materials and the lesion has a characteristic offensive odor \((23, 21)\). Moreover, the lesion was concentrated in hind limbs more than in forelimbs and this feature was agreed with \((4,10,22)\) Table (3).

![Figure 2: Number and percentage of positive samples and site of lesion on feet in sheep and goat. * matched both tests (+,+). 2X= 0.08 , P>0.05](image)

The highly occurrence of foot rot lesion with the scores (3,4 and 5) were tend to be undetectable lesions in the early stage of infection in animals which led to progress the lesions in deep structures of claws and caused severe inflammation and abscessations; so, there was decreased in the productivity and fertility of animals and this feature was agreed with previous study by \((31,12)\).

**Table 3: Number and percentage of positive samples location lesion on limbs in sheep and goat.**

<table>
<thead>
<tr>
<th>Type of animal</th>
<th>No.</th>
<th>Fore limbs</th>
<th>Hind limbs</th>
<th>Total +ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>40</td>
<td>9</td>
<td>16</td>
<td>25</td>
</tr>
<tr>
<td>Goat</td>
<td>40</td>
<td>5</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>14</td>
<td>24</td>
<td>38*</td>
</tr>
<tr>
<td>percentage</td>
<td></td>
<td>% 36.8%</td>
<td>63.2%</td>
<td>47.5%</td>
</tr>
</tbody>
</table>

* matched both tests (+,+). 2X= 0.022, P>0.05

**Table 4: Number and percentage of positive samples of score for sheep and goat.**

<table>
<thead>
<tr>
<th>No. of score</th>
<th>NO. of Sheep</th>
<th>%</th>
<th>NO. of goat</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score 1</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Score 2</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Score 3</td>
<td>11</td>
<td>44</td>
<td>9</td>
<td>69.2</td>
</tr>
<tr>
<td>Score 4</td>
<td>9</td>
<td>36</td>
<td>3</td>
<td>23</td>
</tr>
<tr>
<td>Score 5</td>
<td>5</td>
<td>20</td>
<td>1</td>
<td>7.8</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>65.7%</td>
<td>13</td>
<td>34.3%</td>
</tr>
</tbody>
</table>
Microbiological detection of *F. necrophorum* *sub necrophorum* in infected animals revealed the presence of this causative agent in 46 out of 80 (65.5%) in affected sheep and goat with foot rot and 34 out of 80 (45.5%) of the other sheep were revealed negative results table 1, this results were disagreement with the results of 20 who found that 4 positive samples with *F. necrophorum* was isolated from 84 sheep have been infected with foot rot.

After 48 hours of anaerobically incubation of Colombia blood agar culture plates at 37°C; the colony morphology of *Fusobacterium necrophorum* *sub necrophorum* were appeared as round, grey, and shiny in appearance with diameter about 1-5 µm as demonstrated in (Figure 2 a) while on normal blood agar the colony morphology appeared as tiny round colony have convex surface and some of them were hemolytic but the most of them were non-hemolytic colonies as demonstrated in (Figure 2 b).

![a- Blood agar](image1) ![b- Colombia blood agar](image2)

*Figure 2: Colony morphology of Fusobacterium necrophorum on a- Blood agar and b- Colombia blood agar.*

The grams staining of bacterial smears have been showed the characteristic bacterial morphology of *Fusobacterium necrophorum* and they were observed as gram-negative, long, non-branched filamentous pleomorphic bacilli rang about 100µm in diameter with parallel sides and blunt or tapering ends. 4

The result of PCR technique for detection of *Fusobacterium necrophorum* *sub necrophorum*, leukotoxin gene (*lktA*), in (80) foot rot swab samples of the affected sheep for the extracted DNA from all the isolated bacteria that electrophorised with 1.5 % agarose gel have been showed positive samples with *Fusobacterium necrophorum* *sub necrophorum*, leukotoxin gene (*lktA*), in analysis appeared as single band under the U.V light with the length 403 bp. (Figure 2) of amplified DNA of all strains of *Fusobacterium necrophorum* *sub necrophorum*. 
PCR technique revealed amplified genomic DNA for positive isolates of leukotoxin gene (lktA) of *Fusobacterium necrophorum* *sub* necrophorum in 1.5% agarose gel electrophoresis at the wells no. (1,2,3,4,5,6) revealed positive DNA samples with the length of bands 403 bp. and M = 100 bp. DNA ladder for the swab samples of sheep with Foot rot.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Department of Veterinary Medicine/ Veterinary Medicine College/ University of Kerbala, Iraq and all experiments were carried out in accordance with approved guidelines.

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Do Attendances to the Medico-legal Directorate in Baghdad Referred as Alive Police Cases Have HIV Infection?

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Abstract

Background: This is the first study in Iraq that was carried on alive police cases referred to be examined in the medico-legal directorate (M.L.D.) of Baghdad such as victims of violence and sexual assaults for HIV infection. Reaching subgroups of population that have risky behavior for HIV infection is difficult for the time being in our community due to reasons related to culture, religion &security state

Objectives: To find the prevalence of HIV infection among the attendance to the M.L.D. of Baghdad referred to as alive police cases.

Method: This study had involved (76) cases (45) were males and (31) females among various alive police cases that referred to M.L.D. of Baghdad during the period from 1\1\2011 till 31\10\2011. A written agreement had been taken from all involved individuals. Blood samples were taken, centrifuged, deeply freezed, and tested in specialized HIV laboratory using ELYZA technique EIA Test Kit Laboratories Tes Forsight Acon USA lot; 1105010.

Results: All of the (76) tested samples were negative for HIV infection.

Conclusion: The results of this study supported the general impression that Iraq considered as a low prevalence country to HIV infection, even in high risk groups.

Key Words: Medico-legal directorate, Alive police cases, HIV infections.

Introduction

Many forensic situations involve drug abusers and persons with promiscuous sexual behavior, where the statistical risk of HIV and hepatitis infection is markedly greater than in general population. This poses a risk to pathologist and laboratory staff. ¹

In 1981, a new disease syndrome appeared in human populations in the United States and elsewhere characterized by a deficiency in the immune system (²,³).

Two years after the recognition of AIDS, the causative agent, a human retrovirus in the lentivirus family, was identified (⁴-⁶). Early observations had indicated this virus was spread through intimate sexual contact (e.g., genital fluids), blood and blood products, and through mother-to-child transmission ⁷. Among the above mentioned examples are medico-legal cases that may be examined by forensic doctors as alive police cases referred from the concerned authorities to the medico-legal centers. ⁸

Acquired Immune Deficiency Syndrome (AIDS) is the late clinical stage of the Human Immunodeficiency Virus (HIV). It weakens the immune system and makes
the human body susceptible to and unable to recover from other opportunistic diseases. Consequently, it may lead to high rates of deaths of certain people and worldwide wreaking devastation, involving millions of people and communities.³⁹

Three decades after the first description of AIDS, an estimated 34.0 million (uncertainty range 31.6–35.2 million) people were living with HIV, 2.7 million (uncertainty range 2.4–2.9 million) had become newly infected with HIV over the previous year, including 390 000 children, and 1.8 million (uncertainty range 1.6–1.9 million) HIV-infected persons died. More than two-thirds of HIV infections, roughly 22.9 million persons, were in sub-Saharan Africa, which also accounted for close to 80% of women and 90% of children living with HIV. South and south-east Asia were home to some 4.0 million HIV-infected persons, and the Americas, including the Caribbean, to about 3.0 million. Although HIV/AIDS has caused appalling mortality in MSM and IDUs, the experience of sub-Saharan Africa has made HIV/AIDS the greatest challenge to global health in modern times. Moreover, there has been a severe secondary epidemic of HIV-associated tuberculosis, with an estimated 350 000 deaths among the 1 100 000 persons affected by both infections in 2011.¹⁰

Despite much progress on understanding HIV epidemiology globally, the Middle East and North Africa (MENA) stands as the only region where knowledge of the epidemic continues to be very limited and subject to much controversy.¹¹

There is considerable evidence on HIV prevalence and risk behavior practices among IDUs (Injecting drug users), MSM, (Men who have sex with men) and FSWs (Females sex Workers) in MENA.¹² And this has also a great concern from the medico-legal point of view.¹³

Patients and Method

The study has been performed in Baghdad, the capital of Iraq in the Medico-legal institute after the achievement of the approval from the appropriate health manager authority.

The study has been started in the beginning of 2011 and extended to the end of October of the same year.

The respondents’ that has been included in this study were alive police cases referred to that institute, sample of five milliliters of blood has been aspirated from each body in concern according to his acceptance and voluntarily, putting in mind freedom and confidentiality, the blood that was aspirated has been separated and kept in deep freeze till transportation maintained by cool box to the HIV lab.

ELYZA technique EIA Test Kit Laboratories Tes Forsight Acon USA lot; 1105010 was implied.

It Important to mention that Medico-legal Institute Receive medico-legal cases from both Baghdad Major sectors; Al-Karkh and Al-Rusafa.

Results

A total of 76 respondents were involved, 45(59.22%) males and 31(40.7%) females as shown in figure (1).

Of the study group, 43(56.5%) were married ,while 33(43.4%) were unmarried as shown in figure(2).

Various medico-legal cases had been tested. physical trauma constituted the most important cause as their cases were 34(44.7%), followed by cases of sexual abuse including virginity examination and homosexual (sodomy) cases, in addition to other causes of referral as shown in table (1).

The result of all samples have been tested were negative to HIV.

![Figure (1): Distribution of the study sample according to sex.](image)

![Figure (2): Distribution of the sample study according to marital status](image)
Table (1): Distribution of participants according to the cause of referral.

<table>
<thead>
<tr>
<th>Type of case</th>
<th>Number and percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical trauma</td>
<td>34 (44.7%)</td>
</tr>
<tr>
<td>Sexual abuse:</td>
<td></td>
</tr>
<tr>
<td>Virginity examination</td>
<td>10</td>
</tr>
<tr>
<td>Homosexual examination</td>
<td>18</td>
</tr>
<tr>
<td>Age estimation</td>
<td>5 (6.5%)</td>
</tr>
<tr>
<td>Child nursing</td>
<td>4 (5.2%)</td>
</tr>
<tr>
<td>Alcoholism</td>
<td>3 (3.9%)</td>
</tr>
<tr>
<td>Psychiatric assessment</td>
<td>1 (1.3%)</td>
</tr>
<tr>
<td>Marriage capability</td>
<td>1 (1.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>76 (100%)</td>
</tr>
</tbody>
</table>

**Discussion**

Iraq is classified as a low prevalence country to HIV infection in the region together with other Arab countries and this was attributed to religious and ethical constrictions and low prevalence of illegal sexual practice. Even those who practice illegal sex have no or rare contact with higher risk people from higher risk areas in the world. Probably the immune state of people in this region has a role in this low prevalence which had been noticed in other microbial and viral diseases such as those affecting the intestinal tract.  

Reaching subgroups of population that have risky behavior is so crucial but it is difficult for the time being due to reasons related to culture of community, religion, and security state, so there is shortage in learning about the groups concerned.

Although there is no accurate data on the number of victims of sexual violence who have been infected with HIV as a result of sexual assault, the risk of HIV infection from sexual violence is relatively low.

This study might help for certain extent particularly most of the respondents’ included in this study having sexual abuse in addition to violence activities as shown in table (1). This study also helps to clarify the important role of forensic practice in this vital subject.

It is important to mention that this study is the first one to be performed on alive medico-legal cases in Iraq so there is no previous study to compare with.

**Conclusions**

Negative HIV testing on cases referred to the Medico-legal institute in Baghdad as police cases supports the general impression that Iraq considered as a low prevalence country to HIV infection. Particularly there were cases having risky behaviors that had been included in the study group. This study is not only supporting the Iraqi surveillance but it penetrates by indirect way the risky subgroups which is not always easy to reach. This study also could be regarded as an initial step to state those laboratories tests used to detect HIV infections as routine or mandatory especially in high risk groups of alive police cases examined in the medico-legal centers in Iraq like those involved in sexual assaults in cooperation with specialized AIDS centers to catch up those cases, as this thing is of great value regarding the preventive measures. In spite of low prevalence of HIV infection in Iraq that we hope it remains, but we are not sure of that.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of Medicine \ University of Baghdad, Iraq and all experiments were carried out in accordance with approved guidelines.

**References**

and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. Science 1984; 224:500–503.


Determination of Copper and Lead in Samples of Fungicides and Insecticides in Diyala Governorate

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Abstract

This investigation included the estimation of heavy metals (copper, Cu and lead, Pb) in four types of pesticides that were been selected randomly from commercial markets in the Governorate of Diyala. Two types of insecticides (Lambada Cyhalothrin 5% EC and Zoro Super) and two types of fungicides (Folicur and Goldtanol 50% SL) were tested. The results showed that the highest concentration of lead was found in the insecticide Lambada Cyhalothrin 5% EC (5.621 Mg. l⁻¹), while the lowest value was recorded in the fungicide Folicur (0.147 Mg. l⁻¹). The highest concentration recorded for copper was found in the fungicide Goldtanol 50% SL (28.323 Mg. l⁻¹) whereas a much lower value was found in the pesticide Lambada Cyhalothrin 5% EC (5.995 Mg. l⁻¹). When a search of pesticides banned in the Gulf Cooperation Council (GCC) was performed it was found that the insecticide Lambada Cyhalothrin 5% EC is among the banned pesticides.

Keywords: Copper and Lead, Fungicides, Insecticides, Diyala Governorate

Introduction

A pesticide is a substance or a mixture of several substances that kill agricultural pests and prevent their propagation. The term “pesticide” refers to toxic chemicals that are spread in the environment of the pest by different means and forms ¹. Pesticides are classified in several ways, some of which are classified according to the targeted pest as fungicide, insecticide or spiders pesticide etc. They can be also classified according to the final form of the pesticide, such as liquid, emulsion, concentrated or wettable. Another criterion for classification is toxicity, where pesticides are classified as highly, moderately or lightly toxic, while they are classified according to the mechanism of action into systemic pesticides and contact-transmitted ². The circulation of pesticides and irrational use of them have caused some undesirable environmental manifestations. In fact, pesticides are elements that contribute to shifting the environmental balance with serious threats to both nature and humans which confirms the urge of their avoidance ³. Shortage in farmers’ knowledge about the risks of pesticides to humans and the environment was previously reported, as well as the poor application of measures necessary to reduce their risk ⁴. Addition of farmers to the use of pesticides was reported to cause countless environmental problems ⁵. As high as 20% of the amount of pesticide used remains for one day after treatment, which may lead to higher rates of colon and stomach cancer according to the report of the World Health Organization, due the consumption of food contaminated with pesticides ⁶. Despite the different components of pesticides, the present study sheds the light on the concentration of copper and lead, which are considered as heavy elements with highest specific density. They exist in different environments at low levels, but increase through the introduction of various industrial and agricultural wastes, leading to increased change in the quality of the environment and damage to the living organisms ².

Materials and Method

Pesticides were taken from the commercial markets at random. Four pesticide brands of two types were studied, including two brands of insecticides, Lambada Cyhalothrin 5% EC and Zoro Super, and two of fungicides, Goldtanol 50% SL and Folicur

1: Pesticides for which heavy metals are estimated

1.1. Lambada Cyhalothrin 5% EC

An insecticide that works by skin contact and through digestive system and has an active effect on
insects that attack fruit leaves and soil surface, including the biting and the sucking insects.

1.2. Zoro Super

An effective pesticide on insects and spiders that is used to treat vegetables, broad plantations, fruit trees, and ornamental plants. It is a concentrated emulsion with an active ingredient called Abamectin with a concentration of 36 g / L.

1.3. Goldtanol 50% SL

A fungal and bacterial pesticide that is used to treat soil and has a soluble liquid form.

1.4. Folicur

A systemic fungicide that is emulsified in water and specialized in the control of fungal diseases of wheat and other crops such as vegetables.

*Method of Digestion of Pesticides

The pesticides were directly digested by placing them in the furnace according to the previously described “graphite tube” method 7.

* Quantification of heavy elements in pesticides

Quantification of the copper and lead in the samples was conducted using the atomic absorption device (AA-700 Atomic Absorption Shimadzu) because of its high precision in the diagnosis of heavy metals.

Results and Discussion

The results in table 1 show the presence of copper and lead in all pesticides used in the current study, with the highest value of the lead element in the Lambada Cyhalothrin 5% EC insecticide (5.621 Mg. Γ⁻¹) whereas the lowest was recorded in the fungicide Folicure (0.147 Mg. Γ⁻¹). Copper concentration showed a highest value in the fungicide Goldtanol 50% SL (28.323 Mg. Γ⁻¹) and a lowest value in the insecticide Lambada Cyhalothrin 5% EC (5.995 Mg. Γ⁻¹). The results demonstrate the presence of copper and lead in all of the tested pesticides. It was reported 8 that lead as well as chemical pesticide residues are serious sources of environmental pollution 9. It was also demonstrated that insecticides and heavy metal compounds such as lead and copper, which are spread in the aquatic environment, are considered as dangerous environmental contaminants. The insecticide Lambada Cyhalothrin 5% EC is a pyrotechnic insecticide that is widely known and highly used in Iraq. The chemical structure of this type of pesticides includes a Cyanide atom (a-cyano-3-phenoxybenzyl 3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate). So far, there is no specialized substance that acts on the toxic antagonism of pyrethroid pesticides, but some sedative drugs that relax muscles and reduce spasm levels can be used 10. It was reported that most of the pollution with heavy metals is resulting from human environmental activities, including pesticides and chemical fertilizers 3. A previous investigation of the effect of heavy metals in Al Hilla River region demonstrated that exhausts of cars, generators and waste materials are sources of lead pollution that affects human health, especially children. Lead can be distributed in the body reaching the brain, liver, and kidneys and is stored in teeth and bones where it accumulates over time. There is no current known level of safe exposure to lead. However, this study and our results propose to include chemical pesticides as main sources of heavy elements 11. It was reported that fungicides that are added to the seeds or the soil are transferred from the roots to the leaves, whereas those sprayed on the leaves are transferred to the inside of the plant 3. This cycle suggests that caution is required when using fungicides, because, in addition to the increased damage that may occur as a result of chemical compounds of the pesticide, there is an accumulation of heavy metals found in these pesticides, which increases the risk and effects of their frequent use. Lead and copper are dangerous elements that are sometimes transmitted from the plant to the body of the consumer (human and animal) through the food chain 12. Lead is dangerous due to its cumulative feature, causing physiological damages such as mental retardation and lack of vital functions. The absorption of heavy metals by plants may vary from one class to another and from one type to another. The absorption of elements depends on many factors, including the movement of elements from the soil to the root, the ability of the elements to transit through the cells of the root epidermis, and the transfer of elements from the epidermal cells to the xylem tissue. These metals are then transferred to the leaves that perform the processes of photosynthesis and food manufacturing for all parts of the plant. At the end, the transmission of these elements takes place from the leaves to the storage tissues such as seeds, roots, and buds 13. Previous reports demonstrated that henna extract can inhibit the radial growth of the fungus in tissue cultured of different varieties of date palm trees 14. The inhibitory effect of the growth of
fungus when using henna leaf extracts may be attributed to the effect of active compounds found in the extracts such as resin, flavones, tannins and glycosides. Those results of henna leaf extract inhibition were consistent with many studies that proved similar effects in inhibiting the growth of other fungi. In addition, studies on the effect of volatile oils from lemon and mandarin showed a complete inhibition of the growth of the fungal pathogens compared with the use of fungicides. Thus, such treatments could become substitutes for chemical pesticides. The use of powdered Cinnamomum cassia bark and bitter orange leaves showed inhibitory effects on the growth of Aspergillus ochraceus isolated from some grains and poultry, leading to reduced negative effects.

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>Concentration Mg. l⁻¹</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lead(Pb)</td>
<td>Copper(Cu)</td>
</tr>
<tr>
<td>Lambada Cyhalothrin 5% EC</td>
<td>5.621</td>
<td>5.995</td>
</tr>
<tr>
<td>Goldtanol 50% SL</td>
<td>0.955</td>
<td>28.323</td>
</tr>
<tr>
<td>Folicur</td>
<td>0.147</td>
<td>16.332</td>
</tr>
<tr>
<td>Zoro Super</td>
<td>0.294</td>
<td>16.591</td>
</tr>
</tbody>
</table>

**Table 1: Concentration of heavy metals lead and copper in Samples of Fungicides and Insecticides**

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of Education for Pure Sciences, Biology. Dep. / Univ., of Diyala, Iraq and all experiments were carried out in accordance with approved guidelines.

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Phytochemical Study and Evaluation of Iraqi Fennel Seed Oil as Antibacterial of Urinary Tract Infection

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1Department of Clinical Laboratory Sciences, College of Pharmacy, Mustansiriyah University,
2Department of Pharmacognosy And Medicinal Plant , College of Pharmacy, Mustansiriyah University

Abstract

Objective: This study were designed by investigation of active compounds of the fennel seeds in Iraqi plant and determined the terpenes compounds by gas chromatography analysis and finally shown the biological activity of the volatile oil of fennel seeds upon different pathogenic bacteria which isolated from patients suffered from urinary tract infection.

Methods: in this study we used different methods and different solvent for extract the active compounds such as clavenger for volatile oil, decoction and Soxhlet for main other active compounds.

Results: The results of this study had shown that bacteria such as Pseudomonas aeruginosa, Streptococcus spp and Salmonella spp. were more resistant to The volatile oil of fennel seed than bacteria such as, Staphylococcus aureus and E. coli. five compounds were identified from the n-hexane extract of volatile oil of fennel seeds. Anethole was in maximum content (43.13 %) in retention time 13.762, followed by Estragole (40.84 %) in retention time 12.062, 1,6, octadien-3-ol ,3,7-dimethyl (3.71%) in retention time 9.937, l-fenchone compounds (3.49%) in retention time 9.645 and the minimum one is d-limonene (1.28%) in retention time 8.435.

Keywords: fennel seeds, antibacterial activity, phytochemical screening, GC analysis

Introduction

Medicinal plants are nature’s gift to human beings to make healthy lifestyle free from diseases, and play an important role to look after our health. They are considered to be much safer and certain cure in the treatment of several conditions. Iraq is one of the countries that consider traditional medicine is very important, Iraq, like to any other country in the world, the use of medicinal plants betake back thousands of years. The strategic location, the climatic state of affairs and geographical diversity make it suitable place for cultivate of many medicinal herbs as source of treatment or protection of many diseases. The Foeniculum vulgare consists one of those plants and consider as one of the most important aromatic plant.

There are many traditional medicine for part used (fennel seeds) of F. vulgare consider as carminative and its mainly used with purgatives. There are similarity between fennel water and dill water both used to correct flatulence of infants (gripe water). The volatile oil or called essential oil one of the most active constituents in fennel seed it’s made as flavoring agent in additional used as medicine which reported it have been many components like estragol, trans-anethole, fenchone, and α-phellandrene. The major active compounds of F. vulgare are some of the most important active constituents such as phenols and phenolic glycosides. The phenolic acids structures like 3-O-Caffeoylquinic acid, 4-O-caffeoylquinic acid, 5-O-caffeoylquinic acid, and the flavonoids like eriodictyol-7-rutinoside and quercetin-3-rutinoside, also isolated others constituents from F. vulgare were Quercetin-3-O-galactoside, kaempferol-3-O-rutinoside and kaempferol-3-O-glucoside. The phenolic compounds which isolated from F. vulgare were associated with the prevention...
of some of dangerous diseases because they are caused by oxidative stress like cancer and inflammation. In many studies reported the volatile oil which isolated from fennel seeds of \textit{F. vulgare} act as antibacterial effect against many pathogens bacteria such as Escherichia coli, Bacillus megaterium and \textit{Staphylococcus aureus}, \textit{E. coli}, \textit{Listeria monocytogenes} and \textit{S. aureus} (12,13).

\textbf{Material and Method}

\textbf{Collection of plant material}

The fennel seeds plant was authenticated by National Iraqi Herbarium, Botany Directorate at Abu-Ghraib. The seeds of the plant was washed and dried in the shade at room temperature at Pharmacognosy and medicinal plants department-college of pharmacy-Mustansiriyah University for (7 days) until crisp and then was grinded by mechanical mills and weighed according to previous studies.

\textbf{Extraction}

The essential oil content of fennel seeds was extracted by hydro distillation method by the use Clevenger apparatus (100 g) of the plant material was hydrodistilled by adding (500 mL) of distilled water D.W. in round flask bottom, the plant was left boiling for three hours; the volatile oil was collected after observing that there is no increase in volatile oil was achieved.

\textbf{Preliminary phytochemical investigation}

The extracts of fennel seeds from Baghdad plants was screened according to standard procedures of qualitative investigation to identify the major classes of natural secondary metabolites such as Tannins, Saponins, Flavonoids, Terpenes, Alkaloids, Anthraquinone glycoside and Carbohydrate (16,17).

\textbf{Antimicrobial screening}

The essential oil of fennel seeds was tested for antimicrobial activity of six different pathogenic bacteria which were \textit{Staphylococcus aureus}, \textit{Streptococcus spp.}, \textit{Pseudomonas aeruginosa}, \textit{Salmonella spp.}, \textit{Serratia} and \textit{E. coli} which isolated from patients suffered urinary tract infections by using diffusion well agar method.

\textbf{Results and Discussion}

\textbf{Phytochemical study :}

The preliminary investigation results of active compounds were referred to different active compounds presented according different solvents were used in this study. Ethanol 70% extract was contained Carbohydrate, tannin and flavonoids. while terpene investigated with hexane extract [table1]. Also used Decoction method with water as a solvent and Ethanol 70% by Soxhlet apparatus extract was investigated the others active compounds.

The GC-MS analysis of the individual extracts indicated a complex mixture of constituents of different chemical classes. five compounds were identified from the n-hexane extract of volatile oil of fennel seeds [table 2, figure 1]. Anethole was in maximum content (43.13 %) in retention time 13.762, followed by Estragole (40.84 %) in retention time 12.062, 1.6, octadien-3-ol, 3,7-dimethyl (3.71%) in retention time 9.937, 1-fenchone compounds (3.49%) in retention time 9.645 and the minimum one is d-limonene (1.28%) in retention time 8.435 [Table 2].

The volatile oil of fennel seed possessed antimicrobial activity 0.5, 0.25, 0.125, 0.06 and 0.03 concentration against all microorganisms were used in this study table (3) and the value of the concentration activity was reach to 20, 18, 18, 24, 20 and 12 as inhibition zone at 0.5 concentrated against \textit{Staphylococcus aureus}, \textit{Streptococcus spp.}, \textit{Salmonella spp.}, \textit{Serratia}, \textit{E. coli} and \textit{Pseudomonas aeruginosa} compared with ciprofloxacin as control which gave the higher activity and reach to 24 at 0.5 concentrated [table 3]. The MIC values for The volatile oil can vary depending on factors such as: chemical compositions differences between herbs collected in different countries (differences in the climate, soil composition, age and vegetative cycle stage), as well as differences in strains of microorganisms used (standardized or clinical isolates). The results of this study had shown that bacteria such as \textit{Pseudomonas aeruginosa}, \textit{Streptococcus spp} and \textit{Salmonella spp.} were more resistant to The volatile oil of fennel seed than bacteria such as, \textit{Staphylococcus aureus} and \textit{E. coli}. 


Table 1: Qualitative Phytochemical Analysis of the Extracts of

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>+ve</td>
</tr>
<tr>
<td>Saponins</td>
<td>-ve</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+ve</td>
</tr>
<tr>
<td>Terpenes</td>
<td>+ve</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-ve</td>
</tr>
<tr>
<td>Anthraquinone glycoside</td>
<td>-ve</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>+ve</td>
</tr>
</tbody>
</table>

GC-MS analysis

Figure (1) GC-MS chromatographic profile of fennel seeds oil

Table 2: Chemical composition of fennel seeds oil

<table>
<thead>
<tr>
<th>Retention time</th>
<th>Content %</th>
<th>Compounds name</th>
<th>Chemical structure</th>
<th>Molecular weight</th>
<th>Formula</th>
<th>Similarity</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.435</td>
<td>1.28</td>
<td>d-limonene</td>
<td><img src="image" alt="d-limonene" /></td>
<td>136.24 g/mol</td>
<td>C10H16</td>
<td>99</td>
</tr>
<tr>
<td>9.645</td>
<td>3.49</td>
<td>l-fenchone</td>
<td><img src="image" alt="l-fenchone" /></td>
<td>152.23 g/mol</td>
<td>C10H16O</td>
<td>91</td>
</tr>
</tbody>
</table>
Table 2: Chemical composition of fennel seeds oil

<table>
<thead>
<tr>
<th>Chemical Composition</th>
<th>Molecular Formula</th>
<th>Mol. Wt.</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.937 3.71 1,6,octadien-3-ol ,3,7-dimethyl</td>
<td>C10H18O</td>
<td>154.25 g·mol−1</td>
<td>97</td>
</tr>
<tr>
<td>12.062 40.84 Estragole</td>
<td>C10H12O</td>
<td>148.2 g/mol</td>
<td>98</td>
</tr>
<tr>
<td>13.762 43.13 Anethole</td>
<td>C10H12O</td>
<td>148.2 g/mol</td>
<td>98</td>
</tr>
</tbody>
</table>

Table 3: Antibacterial activity of volatile oil of fennel seed

<table>
<thead>
<tr>
<th>Bacterial isolation</th>
<th>Concentration of inhibition zone (mm)</th>
<th>ciprofloxacin (control )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1/2</td>
<td>1/4</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>18</td>
<td>13</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>Serratia</td>
<td>24</td>
<td>20</td>
</tr>
<tr>
<td>E. coli</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>pseudomonas aeruginosa</td>
<td>12</td>
<td>10</td>
</tr>
</tbody>
</table>

Conclusion

The natural extract from medicinal plants which proved to be potentially effective as (volatile oil of fennel seeds ) can be used as alternative preventives naturally against many pathogenic bacterial specially in urinary tract infections

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College Of Pharmacy, Mustansiriya University, Iraq and all experiments were carried out in accordance with approved guidelines.

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Detection Antimicrobial Susceptibility Patterns of Bacterial Species Isolated from Burns and Wounds Infections in Basrah Hospitals

Ghsoon Al-Kanaany
Assist. Prof. Department of biology, College of Science, University of Basrah, Iraq

Abstract

Burn and wound infection is one of the most frequent serious sicknesses caused by pathogen, chiefly by both gram positive and gram negative bacteria. This study was carried out to identify 25 bacteria isolated from burns and wounds surgical belong to the species: (Staphylococcus aureus, Staphylococcus lentus, Staphylococcus intermedius, Staphylococcus lugdunensis, Pseudomonas aeruginosa, Pseudomonas oxyzihabitans, Pantoea spp., E.coli, Rhizobium radiobacter, Ochrobactrum anthropic, Burkholadia cepacia, Sphingomonas paucimobilis, Klebsiella pneumoniae, Aeromonas sobria). Staphylococcus 9(36%) was the most dominant organism, followed by Pseudomonas 6(24%). Antibacterial activity of some antibiotics was investigated against bacterial isolates. the data demonstrate the most commonly isolated from burns and wounds surgical were Staphylococcus followed by Pseudomonas. All bacterial isolates revealed high resistance to antibiotics were used, Whereas Staphylococcus aureus revealed resistance for three antibiotic: Amikacin, Nitazoxanide, Neomycin. Pseudomonas aeruginosa revealed resistance for four antibiotic: Neomycin, Amoxicillin/Clavulanic acid, Cefotaxime. Pantoea spp revealed resistance for five antibiotic: Neomycin, Trimethoprim, Amoxicillin/Clavulanic acid, Cephalothin, Cefotaxime. E.coli revealed resistance for four antibiotic: Neomycin, Trimethoprim, Amikacin, Cephalothin.

Key words: Staphylococcus; wounds; infections; Neomycin

Introduction

Burn wound infections are a major medical problems in all areas of the world. They can be caused by heat, scalds, electricity, chemical agents. Infections of burns caused by pathogenetic bacteria is one of the most frequent hospital problems in world, especially in modern countries. Hospital acquired infection in burn patients might be caused by exogenous or endogenous. Exogenous infection is obtained during exposure to the hospital workers or medical devices, hospital environment, while Endogenous infection is induced by microorganisms present as the normal flora of the patients. Infection is still source of mortality and morbidity in burn patients. It is assessed that approximately 75% of the mortality associated with burn infections is related to sepsis particularly in modern countries.

Burn injury is one of the most frequent and destructive forms of trauma. Patients with serious thermal injury require specialized care to minimise mortality and morbidity. Human skin surface is the main layer that represents the natural protection of the body tissues from the invasion of potential pathogens, and the occurrence of burns or injury in the skin can lead to destroy and destruction of these tissues and may happen infections of bacteria transmitted to the blood and internal tissues, which is a proteins-rich environment and encourage the growth of microorganisms that play an important role in the pathogenicity.

Burn injuries of patients are high risk of infections for a variety of reasons. For instance, immune compromising effects of burns, available exposed body surface, prolonged hospital stay, invasive diagnostic and therapeutic procedures.

Wounds are defined as a crash in the defensive function of the skin and damage of continuity of epithelium with or without loss of underlying connective tissue. Tainted wounds are probably to be more grievous,
allergic and odoriferous, resulting in increased disquiet and malaise for the patient. Surgical wound infection is determined as festering exude from the surgical wound. It is distinguished by inflammation encircling periwound area. Surgical wound infections are the second most common cause of nosocomial infections. Microorganisms that are liable for surgical wound infection cause activation of immune system results tissue destruction and inflammation.

The dominant microorganisms that associated with wounds infections comprise multi-drug resistant gram-positive and gram-negative bacteria, the most common microorganisms are Staphylococcus aureus which from different studies have been found to form 20-40% and Pseudomonas aeruginosa 5-15% .Escherichia coli, Klebsiella spp and Acinetobacter spp. of the nosocomial infection, with infection chiefly following surgical operation and burns. Pseudomonas aeruginosa is one of the significant species among the genus Pseudomonas. P. aeruginosa is widespread distribution in nature, its virulence and its high antibacterial resistance. P. aeruginosa is nosocomial infection pathogen and an opportunistic, that causes diseases in immuno-compromised individuals.

The aim of this study is to isolate and characterize bacterial species causing burns and wounds infections from patients admitted in Basrah hospitals and determination of the antimicrobial susceptibility of bacterial isolates.

Materials and Method

Collection of specimens

Fifty three swabs have been collected from patients of burns and wounds surgical units with different sex and ages and dissolved into 2ml brain heart infusion broth media after then transported to bacteriological lab and incubated at 37°C for 48 hours. Transported samples comprise 25 post operative wounds and 28 burns from two hospitals: Al-Fayhaa General hospital, Al-Basrah General hospital.

Isolation and characterization of bacterial isolates

The swabs sticks used for collection of specimens were streaked on Nutrient, MacConkey, Blood and Mannitol salt agar and incubated overnight at 37°C. Growth of bacteria have been identified by appearance of colonies, blood hemolysis, microscopic examination by Gram stained. Then bacterial isolates were identified at level of species by using Vitek-2 compact was performed with ID-GN, ID-GP cards, according to the manufacturer’s instructions.

Antimicrobial Susceptibility tests

The antimicrobial susceptibility tests have been accomplished as present in Kirby–Bauer (1966) technique using nutrient agar and different single antimicrobial discs fitted out commercially. Zones of inhibition around the discs have been measured by millimeter (mm) using a metric ruler as present in clinical laboratories standards institute (2011). The antibiotics tested were: amikacin30μg, cefotaxime30 μg, ceaphalothin 30μg, tobramycin 10μg, trimeth- oprim5μg ,neomycin30 μg, nitazoxanide30μg, imipenem 10μg ,meropenem 10μg, amoxicillin/clavulanic acid20/10μg,nalidixic acid30μg.

Results

Out of 53 patients of burn and surgical wounds, 50 cases infected with bacteria. These isolates were identified to Gram negative and Gram positive bacteria by using conventional methods. Whereas the isolates exhibited differential pattern on blood agar and some of isolates ferment lactose when grown on MacConkey media. The identification results with Vitek2 compact system were grouped in fig 1. Among 50 bacterial isolates, 25 isolates identified to species (Staphylococcus aureus, Staphylococcus lentus, Staphylococcus intermedius, Staphylococcus lugdunensis, Pseudomonas aeruginosa, Pseudomonas oryzihabitans, Pantoaea spp., E.coli, Rhizobium radiobacter, Ochrobactrum anthropic, Burkholdria cepacia, Sphingomonas paucimobilis, Klebsiella pneumoniae, Aeromonas sobria).

The study revealed the commonest organism was Staphylococcus with percentage 36% (9 isolates) followed by Pseudomonas 24% (6 isolates), E.coli 8% , Pantoaea 8% with (2 isolates for each), Klebsiella 4%, Rhizobium 4%, Ochrobactrum 4%, Burkholdria 4%, Sphingomonas 4% Aeromonas 4% with (1 isolates for each).

The results demonstrated that the bacteria isolates characterized at species level by Vitek2 compact system was separated into four groups based upon the probability of accurate identification as follows:8(32%) isolates with probability of accurate identification(96-99%),4(16%)


isolates with (93 - 95%), 7(28%) isolates with good(89 - 92%), 6(24%) isolates with (85 - 88%) as show in fig 2.

Antibiotic susceptibility patterns

**Staphylococcus aureus**

The present study showed that *Staphylococcus aureus* exhibited the highest resistance against antibiotics: Amikacin, Nitazoxanide, Neomycin, while they were sensitive to antibiotics Tobramysin, Imipenem as show in fig 3.

**Pseudomonas aeruginosa**

The most effective antibiotics on *Pseudomonas aeruginosa* were Tobramysin, Imipenem while the highest resistance were to Neomycin, Amoxicillin/Clavulanic acid, Cephalothin, Cefotaxime as show in fig 4.

**Pantoea spp**.

The study revealed that *Pantoea spp.* exhibited the highest resistance against antibiotics: Neomycin, Trimethoprim, Amoxicillin/Clavulanic acid, Cephalothin, Cefotaxime while they were sensitive to antibiotics Tobramysin, Imipenem, Amikacin as showed in fig 5.

**E. coli**

In our study, *E. coli* exhibited the highest resistance against antibiotics: Neomycin, Trimethoprim, Amikacin, Cephalothin while they were sensitive to antibiotics Tobramysin, Imipenem as showed in fig 6.

**Antibiotics resistance for Staphylococcus aureus and Pseudomonas aeruginosa**

The results revealed that both *Staphylococcus aureus* and *Pseudomonas aeruginosa* exhibited high resistance against antibiotic Neomycin while both of
them showed sensitive to Tobramycin ,Imipenem.

Discussion

Bacterial infection of wounds is a grave problem in hospital ,chiefly in surgical usage where the place of a sterile operation can become contaminated and subsequently infected 20. In this study , the majority isolated organisms from burns and wounds surgical were Staphylococcus followed by Pseudomonas .the reasons for this high diffusion may be due to factors associated with the acquirement of nosocomial pathogens in patients with long term hospitalization,prior administration of antimicrobial agents, complicating illnesses , recurrent, ,or the immunosuppressive effects of burn these results are not agreement with the work of 18 but agreement with results from previous study 14. Findings from study accomplished at hospital in Nigeria revealed that the most common microorganism were Staphylococcus aureus(25%) and Pseudomonas aeruginosa (20%) 1 . Pseudomonas aeruginosa in this study was resistance to four antibiotics as showed in Fig.4. A primary cause of drug resistance in gram negative bacteria is their ability to generate extended spectrum β-lactamase 19. In results showed that most bacterial isolates exhibited multi drugs resistant. Pseudomonas aerogenosa is adapted to the habitat of hospital due to biofilm construction that provides long survival advantage for the pathogens,and prevent elimination by the host immune system or antibacterial drug treatment 10.

Conclusion

The most common causative agents of wound,burn infections and antibiotics resistance in the present study were Staphylococcus aureus and Pseudomonas aeruginosa respectively,a lso the study observed that Staphylococcus aureus and Pseudomonas aeruginosa were multidrug resistant.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of biology, College of Science, University of Basrah, Iraq and all experiments were carried out in accordance with approved guidelines.

References

Evaluation Protective Role of *Salvia officinalis* Silver Nanoparticles Against Toxic Effect of Bisphenol a in Female Rats

Hussein Abdulmaged Raheem1, Rana Fadhil Mousa2, Ayyed Hameed Hassan2

1MSc. Student, 2Assistant prof., College of Veterinary Medicine / University of Kerbala - Karbala – Iraq

**Abstract**

Bisphenol A (BPA) is a chemical compound found in the environment and used in the manufacturing of plastic, which is known as deleterious part of different body parts. *Salvia officinalis* (SO) is a perennial round shrub in the family of Labiatae/Lamiaceae. It has potent antioxidant and anti-inflammatory effects. The present study was conducted to evaluate the protective role of SO as nanoparticles against harmful effect of BPA in female rats. Eighteen adult female rats were used and divided equally and randomly into three groups as following: first group received DMSO orally and served as control group while second and third groups were gavaged either with BPA alone or in combination with SOSNPs respectively, all the treatments extend 30 days. The result revealed harm effect of BPA by significantly increase the level of estrogen, AST, ALT, ALP, urea and creatinine with significantly reduction in the FSH and LH. SOSNPs show ameliorative effect on all above parameters.

**Keywords**: *Salvia officinalis*, Bisphenol A, FSH, LH

**Introduction**

BPA consider one of the most famous xenoestrogens in the nature before 80 years and enter in many product such as packaging of beverages and food, epoxy, polyvinyl carbon and electronic. Its mimic estrogenic effect via its ability to stimulate estrogen receptors (ER), so its can cause disturbance in the endocrine system. The polymerization increase the risk of exposure, by acceleration the hydrolysis of the ester bond due to heating or acidic environment so the risk of exposure will increase. As a result to frequent wash of the polycarbonate and the temperature may be high or acidic medium, this will increase the risk of hydrolysis. The estrogen receptors are diffused in different organs such as reproductive organs (ovary, uterus, oviduct and ovary) as well as mammary, pituitary, thyroid glands and many other organs, so the binding of BPA with these receptors will lead to increase expression of these cells. BPA has similar effects of diethylstilbestrol on sexual maturity so its banned to use in the baby milk bottles. Nanotechnology is modern technology, this technology focused by the researchers recently due to wide range of applications in energy, electronic purposes as well as in medicine. The nanotechnology recently enter to pharmaceutical line which used in the preparation and development Nano scales particles in the formation of drugs conjugates, Nano crystals, etc. Nanoparticles are cluster of atoms their size range from 1 – 100 nm, due to its size have noble properties as well as morphology and distribution. It have novel properties and functions due to its small size or intermediate, nanoparticles give high efficiency than traditional method for treatment due to mentioned properties as well as decrease the dose and the side effects of chemicals. *Salvia officinalis* rich in canosic acid and rosmarinic acid so it have antioxidant properties, anti-inflammatory, antiproliferative, antibacterial, and anticancer, as well as, sage used as anti-diabetic. Due to aromatic properties the sage used as flavoring agent and antioxidant additive for canned food. *Salvia officinalis* good reducing agent, so it used as reducing agent in the preparation of nanoparticles.

**Materials and Method**

The tested compound is bisphenol A (BPA)

BPA (cas 80 – 05 – 7, > 97%) was obtained from high media company (India), Dimethyl sulfoxide (DMSO) assist as vehicle for suspension of BPA as
well as used as control substance. *Salvia officinalis* herb buy from local market of karbala.

**Animals of study**

This study was carried at the college of veterinary medicine / university of Kerbala, eighteen adult female rats. There ages was ranged from 14 to 16 weeks old and the average of weight (200 – 250 gm). After 15 day of accommodation in metal cages under suitable environment conditions at temperature 23±2 °C and 12 h light / 12 h dark, with ad libitum feeding and water daily, divided randomly into 3 groups 6 animals on each group.

**Experimental design:**

First group administrated 0.25 ml DMSO and, the second group administrated 250 mg / kg BPA and the third group co administrated 250 mg / kg BPA and 150 mg / kg SOSNPs.

**Extraction of salvia officinalis**

After collection of sage plant, wash it by double distilled water (DDW) then cut and grind until powdered it. Weighs fifty grams of powdered SO then mixed with 250 ml from DDW and boiled for 5 minutes, then cooled the aqueous extracts and filtered by filter paper.

**Preparation of salvia officinalis silver nanoparticles (SOSNPs)**

At first prepare 1nM of silver nitrate solution, this solution prepare by adding 0.0170 grms of AgNO3 to 100 ml of DDW by carefully addition, then store in dark colored bottles to avoid auto oxidation after that put the silver nitrate solution and adding it gradually to 10 ml of SO aqueous extract then heat the mixture to 40 C° until see the color from pale yellow to brown.

**Identification of silver nanoparticles**

**Spectrophotometry**

The spectrophotometry was evaluated by using UV-visible double beam (lapomed) were used to identify the stability and formation of silver nanoparticles. The wavelength for this apparatus ranged from (100 – 800) nm, 480 nm was the optimum wave length for SOSNPs solution.

**Scanning Electron Microscope**

By using scanning electron microscope (SEM) (Hitachi), the solution was tested to show the size and shape of the silver nanoparticles. This procedure was done by put one drop of solution on the aluminum – covered by copper grid and films on the SEM system, then let it for 2 minutes, by using blotting paper remove the extra solution then dying the grid.

**Blood sample collection**

At the end of experiment, The animals anesthetized, this done by putting the rats in closed jar containing soaked by chloroform. Then the blood collected by heat puncture. the blood gelled tube and centrifuged for 15 minutes (300 RPM). The supermatant serum was drawn and kept by eppendorf tubes and store at (-4 C°) until analyzed.

**Statistical Analysis**

The results was collected and analyzed by SPSS program version 13, the mean and standard error were dependent in this analysis.

**Hormonal assay**

The (FSH, LH and estrogen) hormones was evaluated at the end of experiment. Assessing the concentration of FSH and LH in the blood is a valuable tool in diagnosing equilibrium in regulating fertility across the pituitary-pituitary gonadal axis. As well as, estrogen evaluation generally considered useful tool in the diagnosis follicular growth and development. The FSH, LH and estrogen concentration was evaluated according to ELISA kit of Monobind incorporation (USA).

**Biochemical tests**

A- **Liver enzymes**

Liver enzymes (AST, ALT and ALP) were determined to evaluate the toxic effect of BPA on the liver and the protective effect of SOSNPs, these tests was done by ELZA depending on the (Agappe diagnostic, spectrophotometer kit, India).

B- **Kidney enzymes**

The kidney enzymes (creatinine and urea) were evaluated to determine the renal toxicity caused by the effect of BPA and the protective effect of SOSNP,
these tests was done depending on ( Agappe diagnostic , spectrophotometer kit , India ) kit 29

Results and Discussion

Synthesis of Silver nanoparticles by using saliva officinalis extract ( SOSNPs ) :

saliva officinalis extract was used to prepare the silver nanoparticles as reductive material , the change in the color is the indicator for silver nanoparticles reduction , this reduction occur due to high content of carnosol, carnosic acid, flavonoid glycosides, niacin, nicotinamide, flavones and estrogenic substances which get it high reductive effect 30 and make the SO suitable for preparation sliver nanoparticles ( 31 , 32 ) .

Identification of silver nanoparticles

The silver nanoparticles were identified by UV identification and SEM scanning . Depending on the optical absorbance of UV radiation ((Labomed Spectro UVD-3200 spectrophotometer ) , the result show the peak of absorption at 480 nm ( figure 1 ) when the color changed to brown this effect by the shape and size of nanoparticles and due to increase in the surface Plasmon resonance ( SPR ) 33 , the change in the color occur due to the reduction of nanoparticles by SO 34 .

Figure ( 1 ) show the peak of wave length for SOSNPs UV test

Figure ( 2 ) SEM image for SOSNP reveal the shape and size of silver nanoparticles
Protective effects of SOSNPs on the reproductive hormones of rats exposed to BPA.

The statistical analysis for the rats reproductive hormones table (1) show significance increase ($P \geq 0.05$) in the estrogen level of the rats exposed to BPA in comparative with control group, due to the effect of BPA at hypothalamus pituitary regulatory circuit by interfering with estrogen receptors ($\alpha$ and $\beta$) in the hypothalamus–pituitary–gonadal axis while in the rats treated with SOSNPs combined with BPA show significance reducing the level of estrogen lower than BPA treated rats to be clear to normal level due to antioxidant properties of SO.

Table 1 statistical analysis for the effect of BPA on reproductive hormones and the protective effect of SOSNPs (Means ± SE).

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>FSH</th>
<th>LH</th>
<th>Estrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>DMSO</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.07±0.04A</td>
<td>2.47±0.09A</td>
<td>47.97±0.26B</td>
</tr>
<tr>
<td>G2</td>
<td>BPA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.77±0.08C</td>
<td>1.27±0.11C</td>
<td>51.82±0.39A</td>
</tr>
<tr>
<td>G3</td>
<td>BPA+SOSNP</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.59±0.05B</td>
<td>2.03±0.07B</td>
<td>48.29±0.55B</td>
</tr>
</tbody>
</table>

Effect of BPA on the liver enzymes and the protective effects of SOSNPs

The statistical analysis for liver enzyme results table (2) show significance ($P \leq 0.05$) increase in the liver enzymes (AST, ALT and ALP) as effect of BPA administration in comparison with control group, these enzymes were tested to evaluate toxicity in the liver, which leakage into the tissue. The toxic effect of BPA come from oxidative stress effect, in addition to cellular damage which come from reactive oxygen species (ROS) this may lead to increase in the liver enzymes level.

Table 2 statistical analysis for the effect of BPA and the protective effect of SOSNPS on liver enzymes (Means ± SE).

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Creatinene</th>
<th>Urea</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>DMSO</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.40±0.014C</td>
<td>28.08±0.35C</td>
</tr>
<tr>
<td>G2</td>
<td>BPA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.72±0.008A</td>
<td>37.59±0.54A</td>
</tr>
<tr>
<td>G3</td>
<td>BPA+SOSNP</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.52±0.011B</td>
<td>31.16±0.46B</td>
</tr>
</tbody>
</table>

- The different litters refer to the significant change between groups.

- No: 3
Conclusion

From the result of present study we conclude that the BPA cause deleterious effects on liver, kidney and reproductive hormones and SOSNPs reduced the harmful effects of BPA.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Veterinary Medicine / University of Kerbala - Karbala, Iraq and all experiments were carried out in accordance with approved guidelines.

References


Assessment of the Needs among Families of Martyrs of Terror Victims in Al Furat Al awsat Governorates / Iraq

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Abstract

Background: Terrorism is a global phenomenon that does not concern religion without religion, sex without sex, land without land or color without color. Who ever monitors the current world situation finds terrorism in various countries of the world and in many.

Methodology: A cross sectional descriptive design study was carried out among families of martyrs of terror victims the period from 31th July 2018 to 29th July 2019. The sample of the study was non-probability "convenient sample" (n=550) of Families Martyrs of Terror Victims in Al Furat Al awsat Governorates. Data are collected Through the use of the questionnaire instrument. Data were analyzed through the application of Statistical Package for Social Sciences (SPSS) program.

Results: The results of the present study show that the the highest percentage of families refers that they resident in city center (25.6%). most of these families expressed an accepted level of such needs (62.6%) and (27.6%) of them expressed an insufficient level of administrative needs. Conclusion: the study concludes that the Most of martyrs’ families expressed moderate level of psychological needs. The majority of these families expressed a lack of support for their social needs. Most of these families have poor monthly income which in turn increase their financial needs.

Conclusions: the Most of martyrs’ families expressed moderate level of psychological needs. The majority of these families expressed a lack of support for their social needs. Most of these families have poor monthly income which in turn increase their financial needs. A small proportion rated the healthcare services they receive as sufficient. Most of these families rated the legal and administrative services as acceptable. Most of these families rated the overall services as insufficient.

Keywords: Assessment, Family, Need, Terrorism and Terror victim.

Introduction

Family is a multidimensional unit performing various functions in a society. It has been a great interest for sociologists to define it on the basis of its various aspects like size of the family, functions of the family, relationship between the family members etc.¹ At the same times families can be classified as nuclear or small family and joint or extended family. The size of the nuclear family depends on the number of children in the family whereas number of uncles, aunt, cousins and grandparents decide the size of joint or extended family.² From the bottom of Maslow hierarchy upwards, the needs are: physiological, safety, love and belonging, esteem and self-actualization. Needs lower down in the hierarchy must be satisfied before individuals can attend to needs higher up. Model of the five-stage can be divided into deficiency needs and needs for growth.³ Terrorism is frequently recognized as referring to acts of violence targeting civilians for political or ideological purposes. In legal terms, although a extensive definition of terrorism has yet to be adopted by the international community, current declarations, resolutions and universal "sectoral" agreements on particular elements of terrorism describe certain acts and key elements.⁴ Victims of terrorism have significant needs which are: the need of being respected, recognized and supported (through data, mental, psychological, practical, expert and involvement in peer support organizations), safety
need (physical, protection from secondary victimization and media victimization), the need of having access to justice and the right to receive compensation from the member State. Victim needs should be evaluated and victims should be notified of their right to access support facilities and aided in the arrangement of suitable referrals where necessary. The use of mass and social media and other support or community-based organizations can be highly helpful and efficient in identifying and informing victims of particular support facilities and how to access them. From the Statistical Information Facility (TIS) compiled by the Counter-Terrorism Center, part of the National Intelligence Director's office, gathered through the Global Tracing System (WITS), the complete amount of terrorist attacks reduced by 14% in 2015 compared to 2014 by 13%. “This is largely due to the decrease in the number of attacks and deaths in Iraq, Pakistan and Nigeria. This marks the first decline in total terrorist attacks and deaths worldwide since 2012.” Terrorism is one of the most serious problems of the present century, and one of the most important phenomena currently affecting human societies, because it has negative effects on the progress and prosperity of nations. An accepted extensive definition of terrorism has never been developed by the international community. Terrorism consider to be the most common problems in Iraq as a developing country. Therefore, the research to find out those families who need to try such care in service that supporting them economically as well as covering them with health services. The present study go which counted the study aided very the needs among families of martyrs of terror victims in Al Furat Al awsat governorates.

**Methodology**

The study aims to assess the needs among families of martyrs of terror victims and to find out the relationship between the family needs with their certain socio demographic characteristics of family such as (age numbers, occupation, marital status, economic status, level of education, family possessions, home sanitation and health care).

**Design of the Study:** The study was a cross sectional descriptive through the period (from 31st July 2018 to 29th June 2019).

**Sample of the Study:** A total of 550 family of martyrs of terror victims in Al Furat Al awsat governorates.

**Study Instrument:** After an intensive review of relevant literatures, the questionnaire is developed and used as a tool of data collection which includes the following:

**Part I:** This part contains of two section socio-demographic family and information of the martyr/injured data; part II assess the needs of the families of martyrs of victims of terrorism.

**Data Analysis:** Descriptive and inferential statistical study tests used through the use of the (SPSS ver-24).

**Results**

The descriptive analysis in this table indicates that the highest percentage of families refers that they resident in city center (25.6%). Regarding number of age, the findings show that (32%) of families having one male with age less than fifteen; the highest percent of families haven’t male age between (16-30) years (35.8%), but (29.3%) of them having only one; families also having only one male with age more than (31) year (41.1%); regarding age of female, (30.4%) of families having two female with age less than fifteen; (33.3%) having one female with age between (16-30) years; and (41.1%) are having two female with age more than (31) years. More than half of families are shown that they are nuclear families (54.7%), and more of them are speaking Arabic (85.1%). Regarding the number of earning family member, (59.8%) having one member among the family. This table reveals that more of martyrs are young adults (75.3%) who are males (68.7%) with intermediate level of education (18.7%), (35.8%) of them are civilian. The social situation for them refers that they are married (44.9%). The relation to martyr/injured family refers that they are father and mother (55.8%).

**Discussion**

The descriptive analysis indicates that the highest percentage of families reported that they live in urban areas (25.6%). This finding is inconsistent with that of the Iraqi Ministry of Planning who reported that the proportion of people who live in rural areas outweigh those who live in urban areas (52.0% vs 48.0%) respectively (Ministry of Planning, 2018a). Regarding number of age, the findings show that (32%) of families having one male with age less than fifteen. This finding is less than that reported by the Ministry of Planning in that the people younger than 15-years is 861.655 who constitute 41.7% of the total population in Babylon.
More than half of families are shown that they are nuclear families (54.7%). This finding is lesser than that of the Ministry of Planning who reported that (95.7%) of families in Babylon are nuclear families (Ministry of Planning, 2018a). Regarding the number of earning family member, (59.8%) having one member among the family. This finding could be explained as that the majority of job opportunities in Iraq are governmental. Concerning the education and occupation of families; the findings reveal that (36.2%) of them reported that less than (50%) of their children are “going or ever gone to school/university”. This finding is less than that reported by the Ministry of Planning who stated that 37.5% of family’s siblings who age 6-years and older have been joining educational institutions in Babylon (Ministry of Planning, 2018a). Around a fifth of fathers are primary school graduates (20.2%). This finding is a little bit lesser than that reported by the Ministry of Planning who reported that 22.3% of population in Babylon are primary school graduates (Ministry of Planning, 2018a). More of families reported that they get health information from social communications (70.9%). The occupation of father indicates that (36%) of father are not working and only (27.3%) has formal working. This finding is higher than that reported by the Ministry of Planning who reported that the percent of unemployment in Babylon is 7.3% in 2017 (Ministry of Planning, 2018a). (50%) of mothers in those families are housewives. This finding is higher than reported by the Ministry of Planning who reported that the percent of housewives in Babylon is 50.0% in 2017 (Ministry of Planning, 2018a). Regarding communication devices, only 0.7% reported that they have telephone. According to the Ministry of Planning, there are 144.434 families who live in the urban areas in Hilla City. Of them, 98.085 have telephones which represents 67.9% (Ministry of Planning, 2018b). With respect to the cellular phones, most of families having mobile phone (99.3%). According to the Ministry of Planning, the total population in Babylon is 2.065.042; of them, 1,791,786 are subscribers for cellular phone lines (Zain, Asiacell, Korek), plus 158,730 who are subscribers for wireless phone lines (Kelemat, Fannos, Iraq communication & post commission (Umniya + Wataniya) (Ministry of Planning, 2018b). Most of them are having television (98.5%). This finding is almost consistent with what reported by the Ministry of Planning in that 98.9% of families in Babylon have TV. With respect to having washing machines, the study findings reported that (84.9%) of families having washing machines. This finding is higher than that reported by the Ministry of Planning in that 78.5% of families in Babylon having washing machines. Most of them have refrigerator (95.8%). This finding is lower than that reported by the Ministry of Planning in that 98.0% of families in Babylon have refrigerators. Regarding car’s possession, only (22.4%) of families having car. This finding is almost consistent with that reported by the Ministry of Planning in that 22.6% of families in Babylon having car(s). Only (4.9%) having agricultural land. This finding is almost consistent with what reported by the Ministry of Planning in that only 1.8% of families in Babylon work in agriculture, hunting, forestry, and fishing (Ministry of Planning, 2018a). Less than half of them have a computer (49.6%). This finding is higher than that reported by the Ministry of Planning in that 22.2% of families in Babylon have personal computers (Ministry of Planning, 2018a). Concerning the monthly income, the study finding revealed that (48.7%) of families having indent income. (66%) of them reported that they receiving support from government. According to the Ministry of Planning, the families in Babylon receive an average of 105.2 Iraqi Dinar from the social care networks (Ministry of Planning, 2018a). The highest percent among families that is (36.2%) reveal that they are living in rented houses. This finding is inconsistent with what was reported by the Ministry of Planning in that 84.2% of families in Babylon live in owned houses compared to those who live in rented houses (6.5%). Regarding the area of homes, (53.8%) of families reported that they live in a home with an area of (50–100) meter. This finding is much higher than that reported by the Ministry of Planning in that 19% of the families in Babylon live in houses of an area of (50–100) meter. With respect to the material from which the houses are built, (58%) of families reported that their houses are built from bricks. This finding is a little bit higher than that reported by the Ministry of Planning in that 56.3% of families in Babylon live in houses that are built from bricks (Ministry of Planning, 2018a).

**Conclusions**

Most of martyrs’ families expressed moderate level of psychological needs. The majority of these families expressed a lack of support for their social needs. Most of these families have poor monthly income which in turn increase their financial needs.

**Financial Disclosure:** There is no financial disclosure.
Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Nursing, Babylon University, Iraq and all experiments were carried out in accordance with approved guidelines.

References
The Psychological, Mental and Physical Effects of Defamation Crimes and Their Severity on the Health of the Iraqi Individual

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Abstract

One of the most serious diseases that affect human health, psychological, mental and physical life is the defamation crimes in the society that have a relationship that affects the individual and directly affects the emotions and feelings and the human mind and kill him and affect human behavior in general. The individual by the press and its impact turns to God acting unconsciously and concentrate in attaining the behavior that he did without realizing the magnitude of the damage caused. **Goals of search:** Psychological, mental, health and physical effects and their severity on human health. Diseases that are dangerous to human mental, physical and mental health in Iraqi society. Prohibitions of publishing defamation crimes in the press that are related to human mental, mental and physical health in Iraqi law. The research community of (100) The sample was adopted at the University of Baghdad (College of Information) as a model has been the research sample (100) of the total research community. The social survey, interview, questionnaire and statistical methods were used.

**Keywords:** psychology, physical effects, crimes, Iraqi individual

Introduction

The press is the fourth authority, this trait has taken it with all merit and power. Why is it an authority because it tickles emotions, activates minds and kills them, it affects human behavior in general¹. The individual is influenced by the press which its influence is transformed into a god acting unconsciously and concentrating in attaining the behavior that he has performed without realizing the magnitude of the damage which inflicted upon him². The problem of research in the crimes of press publication of a special nature has made the legislator not only to punish the writer of the article, but the director of publishing ³ as a pyramid management of the newspaper, this makes proving the judicial responsibility for publishing crimes is very difficult. The author of the article often has an interest to achieve it from publication. The press commits crimes of more than one hundred crime in ten articles, including the permissible and the reduced one ⁴. As well as committing certain crimes that would violate public order and security as a crime of publishing false news that touches on state security or contains a secret of military secrets.

Second: The importance of the research

The press is a collective recognition chair that produces participation because it can paint events for use or not. Crimes are less when newspapers do not talk about them. They do not promote people who are boring and they are services to the government. The press expands to represent the culture and information, which leads to the expansion of the horizons of readers and cultivate in their minds all kinds of knowledge. It plays an important role in detecting corruption, but if it is constrained by legal constraints, it is an obstacle to its functioning.

Third: the goals of the Research

1- Identify the theoretical interpretation that explained the journalistic crime?

2- Identify the most important taboos in publishing in the press in Iraqi law?

3. Identify the most important crimes of the press?
The second topic

Identify scientific concepts and terms

First: The offense

The offense is defined as a crime and it is said that he has committed a crime and his people, and he has committed a crime against them, and to them he has committed a crime and a crime for his family. Books and men earned him an offense. papers that condemn them during the twenty-four hours of the crime(1). It is also known as: is the one who has watched during the occurrence or seen the effects after a period of time in special circumstances limited by the law", but the definition can be avoided in terms of the control of crime and in terms of procedures for the application of the laws of the Code of the "Criminal Procedures"(2).

The offense is defined as procedural: it is the offense that is seen in the course of its occurrence. Its punishment is limited by law that includes all the crimes that arrest the perpetrators(3).

Second: The Journalist

Linguistically the journalist is known: A person works in a newspapers means paper man "Alorak" or "Journalist". Which is quoted from the western label to denote daily newspapers(4).

A journalist is defined as the one who collects information about an event or consequences of that coverage by publishing or broadcasting because its results are often beyond the will of the sender(5).

The journalist also known as: the person who transmits the events that occur in the place where he is sent and the news is conveyed by Powell to the news agency (6).

It has another definition: the person or individual who works in a media company or the individual is working in the press departments in universities or some media organizations which employ these journalists (7).

Third: The Press

Linguistically the press defined as: A source that is derived from the work of newspapers is that the press, it is the art of creating and writing newspapers and magazines (8).

The press defines a convention: In the words of English "Burke": (the press is the fourth authority) (9).

The press is defined as a number of pages that are issued daily or on the dates of the organization of political, economic, social, cultural and related news. that can be one (10).

Fourth: the intellectual

Is the one who has a comprehensive view of the change of society and works for the benefit of all sectors of society and has the ability to critique the social and scientific and agreed person intervenes and nudges his nose in what does not matter (11).

The intellectual is also defined: A person who imposes himself and his presence in society and he does not need to specify because he declares himself through his scientific practice, of confusion (12).

The intellectual also is known as: A part of the social sacrifice that is concerned beyond its craft, relying on it together with the general issues of Hungary. The intellectual is characterized by the neighborhood of cultural adaptation and the ability to learn from othersn (13).

Chapter Three

The first topic: Theoretical interpretation of the research subject

Theory of PH.D. Laila Abdelmajeed tagged (media legislation), 2005.

it works to support the authority that has given it the right to exist, and the freedom of the media under this theory exists to the extent permitted by national leadership at any time (14).

The Press Freedom Committee was invited to a free and responsible press within and outside the United States. The First Royal Committee of the Press was formed in 1949. of this theory, as well as forming a press council (15).

1- The press as well as other media must accept and implement certain commitments to society (16).

2- These commitments can be implemented by adhering to professional standards for the transmission of information such as truth, accuracy and objectivity.
Prohibitions of publishing the press in Iraqi law

First: Violation of internal security: The press must refrain from publishing any news or information or anything that might touch security within the state ideas and encouraging the overthrow of the regime by force and spreading news that frightens people (17).

Second: It is not permissible to harm the interests of the State or States by means of matters that may lead to this (18).

Fifth: Which the law or the investigating authority has decided to ban publication and prosecution at the trial court (19).

Third: the government and may lead to the criminalization and restrict their personal freedom to enter the prison so they will impose the penalty of imprisonment (20).

Third topic: The Crimes of the Press:

but the crimes of public law are concerned with material fact, whether signed in secret or overtly in reverse with press crime that requires public (21).

Press Crimes

First: Crimes of defamation:

1-Ejaculation: It is the attribution of facts or specific things that should be scorned by those who have sought it, he is punished legally if it is correct (22).

Secondly: Crimes of disclosure: are the dissemination or broadcast of news, data, information or documents relating to the secrets of defense. The most important crimes of disclosure are punishable by law (23).

1- The crime of disclosing the secrets of defense: It relates to the dissemination or broadcasting of information related to the armed forces, their formation or equipment, and in general.

2- The crime of disclosing the secrets of the state is related to the dissemination of security and its reasons of publication From these conditions a (24):

1-The news is incorrect.
2-Bad faith of the journalist.

The penalty varies according to the type of news (25):

A- If the intention of publication is to disturb the public peace or to cause panic

Fourthly: Crimes of incitement: is to urge others to commit a particular matter. Creates the design he has to commit to it. And the conditions that make the fair is as a partner in the crime (26).

1-The incitement shall be direct in the sense that the subject of push shall be subject to commit a crime or certain offenses.

2- The crime w committed indeed as a result of incitement.

Chapter Three: Field Research Procedures

A-Community and a sample This community has been identified by the College of media at the University of Baghdad. As for our study, the research community may be (100). The whole sample was adopted from the research society (College of media - University of Baghdad). The research sample was (100) of the total research community (27).

Second: Research hypotheses:

After reviewing the literature that dealt with journalistic crimes, in light of this, the following hypotheses were formulated (28):

1- "That the press is one of the factors fueling violence at different levels."

2-"There is a relationship between sex and the enactment of legal legislation that eliminates the publication of press offenses."

Third: Research areas:

1-The human field: means the individuals to be conducted the field study, and the human area of our research was based on the professors at the College of media at the University of Baghdad.

2-Geographical area: It is intended to determine the place or geographical organization where the field study will be conducted and identified in the professors of the Faculty of media at the University of Baghdad a geographical area for conducting the study.

3-Timetable: This is the period of time taken for the collection of research information and data. The field
study process took place from 8/2/2018 to 1/3/2019.

Statistical methods were used: statistical methods in the process of describing and analyzing research data (29):

1- percentage(%):

\[ \text{Percentage} = \frac{\text{part}}{\text{All}} \times 100 \]

2- Kay box test (Ka 2):

Presentation and analysis of research data

First: First topic: Preliminary data of the research community:

Table (1) shows the gender of the respondents

<table>
<thead>
<tr>
<th>Sex</th>
<th>Num</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>53</td>
<td>53%</td>
</tr>
<tr>
<td>Females</td>
<td>47</td>
<td>47%</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100%</td>
</tr>
</tbody>
</table>

The results of the above table show that the percentage of males was (53%), while females were (47%). We conclude from this that the proportion of males was lower than the proportion of females. This indicates that the community is the majority of males.

Table (2) shows the certificate obtained by the respondents

<table>
<thead>
<tr>
<th>Kind of answer</th>
<th>Num</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Master</td>
<td>54</td>
<td>54%</td>
</tr>
<tr>
<td>Ph.D</td>
<td>46</td>
<td>46%</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100%</td>
</tr>
</tbody>
</table>

We conclude from this that the highest percentage is among the respondents who obtained the master’s degree, reaching (54%) of the research community and the lowest percentage among the respondents who obtained the doctorate degree, reaching (46%) of the research community.

Table (3) shows the most common press crimes

<table>
<thead>
<tr>
<th>Kind of answer</th>
<th>Num</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crimes of disclosure</td>
<td>32</td>
<td>32%</td>
</tr>
<tr>
<td>Crimes of ejaculation</td>
<td>23</td>
<td>23%</td>
</tr>
<tr>
<td>Crimes of incitement</td>
<td>17</td>
<td>17%</td>
</tr>
<tr>
<td>Crimes expose some characters</td>
<td>18</td>
<td>18%</td>
</tr>
<tr>
<td>Other crimes</td>
<td>10</td>
<td>10%</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100%</td>
</tr>
</tbody>
</table>

The crimes of disclosure and 32%, which is higher than those who answered the crimes of slander and slander (23%), as well as higher than those who responded to the crimes of disclosure of personalities and by (18%) they are higher than the crimes of incitement by (17%). Therefore, (10%) of the research community responded to other crimes.

Table (5) shows respondents that the press can play a role in fueling violence

<table>
<thead>
<tr>
<th>Answers</th>
<th>Num</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>70</td>
<td>70%</td>
</tr>
<tr>
<td>No</td>
<td>30</td>
<td>30%</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100%</td>
</tr>
</tbody>
</table>

Highest and that which was (70%) They believe that the press can play a role in fueling violence at all levels, therefore it is higher than the percentage of respondents who (30%) of the research community.

Explains whether the government’s policy is based on some of what the press publishes.

Table (5) shows and Explains whether the government’s policy is based on some of what the press publishes

<table>
<thead>
<tr>
<th>Kind of Answer</th>
<th>Num</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large</td>
<td>74</td>
<td>74%</td>
</tr>
<tr>
<td>Few</td>
<td>7</td>
<td>7%</td>
</tr>
<tr>
<td>Do not affect</td>
<td>19</td>
<td>19%</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100%</td>
</tr>
</tbody>
</table>
We conclude from this that the highest percentage of respondents (74%) which is higher than those who did not respond by (19%). Consequently, the lowest percentage of respondents (7%) responded to the research community.

**The results**

1. We conclude from this that the percentage of respondents who answered that they live in their own houses, who is the highest percentage (50%).

2. The answer with (yes) which the researchers support the enactment of legal legislations that abolish the publication of press offenses, is (56%).

3. We conclude from the answer rate (yes) that the researchers are the ones who believe everything that is said in the press, which was (53%).

4. We conclude that the answer to (yes) that the researchers believe that these crimes affect individuals, which was (78%).

5. We conclude from the answer rate (yes) that the researchers believe that these crimes are a threat to society, which was (87%).

6. We conclude from the answer rate (yes) that the researchers believe that the press plays a role in inflaming violence at all levels, which was (70%).

7. We conclude from the percentage of answer (yes) that the press has a role in fighting crime in all its forms is the highest percentage which was (73%).

8. We conclude from the percentage of the answer (yes) that the researchers emphasize the role of press crimes in the flags of community members of community issues that are committed in secret is the lowest percentage which was (48%).

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Babylon University and all experiments were carried out in accordance with approved guidelines.

**References**

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8. Tarek S. Lessons in Publishing Crimes, Cairo University, Dar Al-Nahda Al-Arabiya, Cairo, 1997.
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New Diaz Coupling Reaction, Cloud Point Extraction Spectrophotometric Determination of Sulphadimidine Soudium in Pure form and Pharmaceutical Preparation with Salicylic Acid as the Coupling Reaction

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Abstract

In this research we study two simple methods, rapid of use spectrophotometric determination of sulphadimidine sodium (SDMS). The first method based on diazotization of drug by sodium nitrite at 5Cº followed by coupling with Salicylic Acid in basic medium to form yellow color. The product was measured at 453 nm. Beer’s law is obeyed in the concentration range of (1-16) μg·ml⁻¹. Sandell’s sensitivity was 0.07575 μg·cm⁻¹, the detection limit was 0.3992 μg·ml⁻¹, and the limit of Quantitation was 1.07879 μg·ml⁻¹. The second method was used cloud point extraction (CPE) with used Triton X-114 as surfactant. Beer’s law obeyed in the range of concentration was (1-12) μg·ml⁻¹. Sandell’s sensitivity was 0.04 μg·cm⁻¹, the detection limit was 0.0259 μg·ml⁻¹, and the limit of quantitation was 0.029563 μg·ml⁻¹. All variables were study including of the reagent concentration, reaction time. The composition of product (1:1). The methods were effectively useful to the determination of SDMS in pharmaceutical dose form, and the attained results were in good agreement with the official and other methods in literature. No interference was observed from the commonly encountered additives and recipients.

Keyword: Cloud Point Extraction, Salicylic acid, Triton X-114, Sulphadimidine Sodium.

Introduction

Sulfa drug (sulfonamide) were the fist antibiotics, discovered in 1935 by Gerhard Domagk (1). Above 5400 products of sulfanilamide were formed, however around 20 products formulas are usually obtainable the structure of sulfonamide (2).

Table (1) : General properties of SDMS :

<table>
<thead>
<tr>
<th>Chemical structure</th>
<th>Nomenclature</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image.png" alt="Chemical structure" /></td>
<td>2,4,6-(Aminobenzensulfonamido- dimethylpyrimidine)</td>
</tr>
</tbody>
</table>
Cont.. Table (1) : General properties of SDMS :

<table>
<thead>
<tr>
<th>Other names</th>
<th>Sulfamethazine Sodium, Sulfadimethylpyrimidine sodium, Sulfamethazine sodium, Sulfadine sodium, sulfodimesin sodium, etc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula</td>
<td>C13H14N4O2SnA</td>
</tr>
<tr>
<td>Molecular Weight</td>
<td>300.312</td>
</tr>
</tbody>
</table>

**Instrumentation and Apparatus:**

**Instruments:**

UV-Vis spectrophotometer: SHIMADZU, Double beam UV-Vis, model UV-1800 made in Japan. The range of wavelength (190-1100) nm, cell quartz with path 1cm., Water Bath : A thermostat water bath, Memmert, made in Germany, Electric Balance: Sartorius (0.0000), made in Germany, Centrifuge: Triup International corp, TRIU 800 Centrifuge, made in Korea & PH meter: HANNA, PH meter, HI 83141.

General procedure for Azo coupling:

The prepared Azo Coupling product are added in volumetric flask (10ml) in ice bath , 1ml of Sulphadimidine Sodium (SDMS) (1000 µg ml-1) ,1ml for hydrochloric acid , 1ml for sodium nitrate (1%) ,1ml for sulphamic acid (1%), 1ml for salicylic acid (1000 µg ml-1) ,at last added 1ml for sodium hydroxide and complete the volume by distilled water .Then absorbance is measured by UV-VIS. And the maximum wave length show in figure:(1-1).

General procedure for CPE:

A typical experiment of cloud point include the following steps: taking the volumetric flask (10ml) and added the optimum condition of azo coupling and added 1ml for surfactant (10%) and complete the volume by distilled water . The contain of volumetric flask transfer to centrifuge test tube then added the mixture in water bath 60 C° at 20 min and separated by centrifugation 4000 rpm at 20 min. Test tube taken in ice bath to increased viscosity micelles layer 1min. then become easily separated . The separated sediment s dissolved by 1ml of ethanol and measured the absorbance by UV-VIS.

**Result and Discussion**

**First methods:** Spectrophotometric determination of sulphadimidine sodium (SDMS) by oxidation coupling reactions .

**Optimization Parameters for Reaction .**

All of the factors that affect to the absorbance of formation of azo dye product are optimized to improve the sensitivity and detection limit for the determination of the drugs .All optimization work under wavelength at 473 nm.

![Figure(1) : Absorbance spectra of the Resulting Dye,SDMS](image-url)
Effect of Acid Type.

In this study, made series experiment using (0.1 – 0.5 and 1 M) of different acid [HCl, H$_2$SO$_4$, HNO$_3$, H$_3$PO$_4$ and CH$_3$COOH] that follow the same procedure that [1 ml of drug SDMS, 1 ml of each acid, 1 ml of NaNO$_2$, 1 ml of H$_3$NSO$_3$, 1 ml salisilic acid and 1 ml of NaOH] in volumetric flask 10 ml and complete the volume by distilled water to formation diazonium salt. After that measuring the absorbance at 453 nm.

It is clear from this study that the phosphoric acid (0.5 M) gives higher absorbance for SDMS, this acid is a few of use in subsequent experiments.

Optimum Volume of 0.5M phosphoric acid.

The same addition for SDMS is [1 ml drug, with varying volumes of 0.5 H$_2$PO$_4$ from (0.1-1) ml, 1 ml NaNO$_2$, 1 ml H$_3$NSO$_3$, 1 ml Salsilic acid and 1 ml of NaOH in 10 ml volumetric flask and complete the volume by distill water. Then measured the absorbance at 453 nm and the optimum volume for higher absorbance were fixed for sequence experiment 0.7 ml.

It is obvious that absorbance increased with increased of the volume of acid, suddenly the absorbance decrease because the primary amine becomes inactive (5).

Base Type.

We used different bases [NaOH, KOH, K$_2$CO$_3$, Na$_2$CO$_3$, NH$_4$OH, NaHCO$_3$] with different concentration (0.1, 0.5 and 1 M) and that follow the addition in 10 ml volumetric flask. Then see the higher of absorbance for the base is KOH 90.5 M)

It is clear the potassium hydroxide give the higher absorbance, this base it is fixed in subsequent (6).

Optimum Volume of 0.5M KOH.

The same addition for SDMS in volumetric flask of 10 ml with different volume of KOH (0.1-1 ml) and complete the volume by distill water. Then the absorbance were measured at 453 nm and higher absorbance that fixed at 0.9 ml.

It is clear that absorbance increased with increased the volume of KOH, but rapidly decrease because this volume was essential to coupling with SDMS (10).

Optimum Volume of 1% Sodium nitrite.

The same additions were added with varying volume of 1% NaNO$_2$ from (0.1-1.5) ml and the absorbance were measured of optimum volume and fixed at 1.1 ml.

It is obvious the absorbance increase when the volume of NaNO$_2$ increase, suddenly the absorbance decrease because the nitrate toxic became a high rate of pollutants affected on diazonium salt (8).

Optimum Volume of 1% Sulphamic acid.

The additions were added of [1 ml for SDMS, 0.7 ml H$_3$PO$_4$, 1.1 ml NaNO$_2$ with varying volume of 1% H$_3$NSO$_3$ from (0.1-1) ml, 1 ml sulisilic acid and 0.9 ml KOH] in volumetric flask 10 ml and the higher absorbance were measured of optimum volume and fixed at 0.4 ml.

In this figure is obvious when the volume of Sulphamic acid increase, the absorbance increase however the absorbance decrease rapidly because this volume remove nitrite and escape as nitrogen gas (9).

Optimum Volume of (100 µg mL$^{-1}$) Reagent.

The same additions are for [1 ml SDMS, 0.7 ml H$_3$PO$_4$, 1.1 ml NaNO$_2$ with varying volume of 1% H$_3$NSO$_3$ from (0.1-1.5) ml, 1 ml salisilic acid and 0.9 ml KOH] in volumetric flask 10 ml and the absorbance of optimum volume were measured 1.1 ml at maximum wavelength and fixed for sequence experiment.

In this graph was clear when the volume of reagent increase, the absorbance increase but, gradually decrease because this volume was essential to coupling with SDMS (10).

Reaction Time on Stability Color Product.

When the optimum volumes of parameters were complete we study effect of stability color product of SDMS [1 ml SDMS, 0.7 ml H$_3$PO$_4$, 1.1 ml NaNO$_2$, 1 ml 1% H$_3$NSO$_3$, 1.1 ml salisilic acid and 0.9 ml KOH] in volumetric flask 10 ml. The stability of product was one of significant factor that diazotization and clouding reaction were depended on it, as a result the time needed was (0-60) min. Then the absorbance were measured and fixed the high reader at high maximum wavelength.
This apparent the best time of product stay stable for SDMS was 50 min \(^{(11)}\).

**Order Addition.**

All additions of diazotization and coupling reaction are added for SDMS with optimum condition. Then followed diluted by different polar solvent [ water , ethanol , methanol ,1- propanol ,acetonitrile & acetone ] in volumetric flask 10 ml ,at maximum wavelength for each drug the absorbance are measured and recorded for the best solvent.

It is obvious the greatest addition is the order two because it is give the higher absorbance \(^{(12)}\).

**Type of Solvent :**

All the compounds of the optimal parameters for SDMS were dissolved in volumetric flask and completed this volume for different solvent to study the best of solvent and measured the absorbance.

In this experiment shown the best of solvent was water because gave higher absorbance. It is sensitive, no expensive, economically and nontoxic\(^{(13)}\).

**Temperature:**

In this experiment study the effect of different temperature on the color product have been (5-60 °C). the absorbance were measured and the higher absorbance was recorded.

It is clear the best temperature is 20°C because was given the higher absorbance \(^{(14)}\).

**Stoichiometric Determination of Product. :**

**Continuous Variation Method** \(^{(15)}\).

A series of different volumes of reagent and drug are prepared (0.1-0.9) ml ,with concentration \((4\times10^{-4} \text{ M})\) in volumetric flask 10 ml .The additions are optimal condition and complete the volume by distilled water \(^{(10)}\). Then absorbance are measured by UV-VIS at \(\lambda_{\text{max}} =473\) nm . the stoichiometric ratio between reagent[R] and drug[D] result 1:1.

**Mole Ratio Method**

In this method the volume of drug is fixed at 1 ml with concentration \((4\times10^{-4}\text{M})\) and the volume of reagent is change (0.5-4.5 ml ). The optimum of addition is complete by distill water in volumetric flask 10 ml and the absorbance is measured by UV-VIS at \(\lambda_{\text{max}} =473\) nm. The stoichiometric ratio between reagent[R] and drug[D] result 1:1.

**3- Calibration Curve for complex of SDMS - α-Naphthol .**

In this study the solution are prepared in volumetric flask 10 ml continue of different volume of SDMS (1-12) µg mL\(^{-1}\) by taken [0.7 ml \(\text{H}_3\text{PO}_4\) , 1.1 ml \(\text{NaNO}_2\) ,0.4 ml \(\text{H}_2\text{NSO}_3\) , 1.1 ml \(\text{Salicylic acid}\), 0.5 ml KOH ]. The volume complete by distill water and measured the absorbance by UV-VIS at maximum wave length against a blank solution prepared same condition without drug . Linear calibration graph is established by blotting absorbance against concentration of SDMS ,it found (1-12) µg mL\(^{-1}\) obeys the Bear Law . The molar absorption coefficient of product equals \((9.970\times10^3\text{L}.\text{mol}^{-1}.\text{cm}^{-1})\) and sandal’s sensitivity (0.03012 µg mL\(^{-2}\)).

**3- Effect of interference.**

In this study effect of interference expected present with SDMS by added 1ml (1000 ppm), and the rest of optimum addition in volumetric flask 10 ml and complete by distill water. Then measured the absorbance by UV-VIS.

<table>
<thead>
<tr>
<th>NO.</th>
<th>100ppm interference</th>
<th>Abs.</th>
<th>Recovery %</th>
<th>Erel%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lactose</td>
<td>0.162</td>
<td>103.7878</td>
<td>3.78</td>
</tr>
<tr>
<td>2</td>
<td>Starch</td>
<td>0.171</td>
<td>110.606</td>
<td>10.606</td>
</tr>
<tr>
<td>3</td>
<td>Arabic Gum</td>
<td>0.156</td>
<td>99.2424</td>
<td>-7.6</td>
</tr>
<tr>
<td>4</td>
<td>Glucose</td>
<td>0.160</td>
<td>102.2727</td>
<td>2.272</td>
</tr>
<tr>
<td>5</td>
<td>Tale</td>
<td>0.159</td>
<td>101.515</td>
<td>1.515</td>
</tr>
<tr>
<td>6</td>
<td>Tri methyprine</td>
<td>0.161</td>
<td>102.78</td>
<td>2.98</td>
</tr>
<tr>
<td>7</td>
<td>Without interference</td>
<td>0.158</td>
<td>100.7575</td>
<td>0.757</td>
</tr>
</tbody>
</table>

This result show in table (1-13) there is no interaction between interference and SDMS \(^{(16)}\).
4- The Stability Constant of Color Product.

The stability constant K show in the table (1-14).

Table (3): Data of The Stability Constant of Color Product of SDMS.

<table>
<thead>
<tr>
<th>Volume of 4x10-4M of SDMS / ml</th>
<th>Final con. SDMS /M</th>
<th>As*</th>
<th>Am*</th>
<th>A</th>
<th>K (L..Mol-1)</th>
<th>Mean of K (L..Mol-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3</td>
<td>1.2x10-3</td>
<td>0.064</td>
<td>0.066</td>
<td>0.03030</td>
<td>3.1811X106</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>2x10-3</td>
<td>0.093</td>
<td>0.096</td>
<td>0.03123</td>
<td>2.99 X105</td>
<td></td>
</tr>
<tr>
<td>0.7</td>
<td>2.8x10-3</td>
<td>0.121</td>
<td>0.123</td>
<td>0.016260</td>
<td>0.999 X106</td>
<td></td>
</tr>
</tbody>
</table>

It is clear the stability constant is high, so the dye formed is very stable.

Am = the high absorbance, As = the few absorbance.

5- Accuracy and Precision Test.

The table (1-15) show the accuracy and precision of SDMS, which study at different concentration (12, 9, 6, 3). It is clear this result has a good accuracy and precision.

6- Application:

The proposed method applied on [Montajat Pharmaceuticals. Saudi Arabia] injection (SULJAT) that contains (200mg) from Sulphadimidine Sodium in 100ml. The result is good quality and summarize in table (1-16).

Table (4): Data of Accuracy and Precision Test.

<table>
<thead>
<tr>
<th>Amount of SDMS / µg mL-1</th>
<th>*Found</th>
<th>Recovery %</th>
<th>Average Recovery %</th>
<th>Erel%</th>
<th>Average Erel%</th>
<th>RSD%</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>11.8179</td>
<td>98.482</td>
<td>99.5017</td>
<td>-1.5175</td>
<td>0.9630</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>9.12118</td>
<td>101.3464</td>
<td></td>
<td>1.3465</td>
<td>0.2525</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>5.0605</td>
<td>101.2116</td>
<td></td>
<td>1.2116</td>
<td>1.9514</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.9090</td>
<td>96.9668</td>
<td></td>
<td>-0.0303</td>
<td>4.3554</td>
<td></td>
</tr>
</tbody>
</table>

*= Average for five determination.

Second Method: Spectrophotometric determination of Sulphadimidine Sodium (SDMS) with using Cloud Point Extraction Technique.

Effect Type of Surfactant with SDMS.

The surfactant have an important part in cloud point extraction process. The basic of practical depended of micells for extraction. In this experimental we added all the last parameters with added 1 ml of different surfactant in volumetric flask 10 ml and the volume completed by distilled water at 60°C for 20 min. then by centrifugated separated at 4000rpm for 20 min and dissolved in 1 ml ethanol and considered by UV-VIS at \( \lambda_{\text{max}} = 453 \text{nm} \).
The Volume of Triton X-114.

Effect of Equilibrium Temperature.

In series used varied temperature (35-70°C) for 20 min. to formed cloud point and separation by centrifugation at 4000rpm for 20 min then dissolved by 1ml ethanol and measured by UV-VIS at $\lambda_{\text{max}}=453\text{nm}$ and fixed.

The best of Temperature were fixed at 50°C.

The Incubation Time.

The solution prepared of all the parameter and the incubation time for (5-35min) to form cloud point and separated by centrifugation then measured by UV-VIS at $\lambda_{\text{max}}=453\text{nm}$.

The best of incubation time is 20 min.

Preparation of Calibration Curve in CPE.

The set of experimental prepared by increasing concentration of SDMS (1-12 $\mu$g mL$^{-1}$) in volumetric flask 10 ml and the volume complete by distilled water then measured by UV-VIS at $\lambda_{\text{max}}=453\text{nm}$.

Application:

The proposed method applied on (silphdimidine sodium33.3% injection . Jorden). That contain (33.3 gm each 100gm). The result is good and summarized in table (5).

<table>
<thead>
<tr>
<th>Amount of SDMS / $\mu$g mL$^{-1}$</th>
<th>*Found</th>
<th>Recovery %</th>
<th>Average Recovery %</th>
<th>Erel%</th>
<th>Average Erel%</th>
<th>RSD%</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>11.968</td>
<td>99.7333</td>
<td>99.6444</td>
<td>-0.0266</td>
<td>-0.1155</td>
<td>0.2796</td>
</tr>
<tr>
<td>9</td>
<td>8.968</td>
<td>99.6444</td>
<td></td>
<td>-0.3555</td>
<td>-0.1155</td>
<td>0.3336</td>
</tr>
<tr>
<td>6</td>
<td>5.952</td>
<td>99.2</td>
<td></td>
<td>-0.08</td>
<td>-0.1155</td>
<td>0.5622</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>100</td>
<td></td>
<td>0</td>
<td>0.00</td>
<td>1.1925</td>
</tr>
</tbody>
</table>

* = Average for five determination.

Table (5): Data of Accuracy and Precision Test.

Conclusion

The method of Cloud point extraction is demeans, good, safe and use pre-concentration technique to determine Sulphadimidine Sodium by UV/VIS. In designed method is a gentleness, selectivity and gave a low limit of detection and good RSD.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of chemistry, College of science for women, University of Baghdad, Iraq and all experiments were carried out in accordance with approved guidelines.

References


The Relationship of Refereeing Performance of the Leading Personality and Thyroxin Hormone Level for Football Referees (First Division)

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¹Assistant prof., University of Kufa/College of Physical Education and Sport Sciences / IRAQ, ²Assistant prof., University of Kufa/College of Physical Education and Sport Sciences / IRAQ, ³Assistant lecturer, University of Kufa/College of Education for Girls / Department of Physical Education and Sports Sciences / IRAQ

Abstract

Refereeing has an important part in the dictionary of football game, developing the referees’ abilities and prepare them in a suitable way with the development of the nature of competition between the teams in order to enhance their abilities to make the right decisions that achieve the justice. Thus, the referee and player are the same, both of them needs to the correct preparation in physical, dynamic, knowledge and mental aspects as well as other important personal characteristics such as: leading ability, self-confidence, emotional balance… etc., undoubtedly, refereeing has an essential role in developing football, so when the referees’ level increase that leads to increasing the level of competition. The value of the present research lies in identifying the relationship between refereeing performance, the leading personality and thyroxin hormone level for football referees (First Division), which may be contribute in the performance of referees in a stadium so through these things, referee’s level can be predicted as it achieves high results in these variables. The researchers used the descriptive method of correlation and predictive relationships because of its appropriate to the problem of referees (first division), for the academic year 2018, their number is (15) referees.

Keywords: Leading Personality, Refereeing Performance and Thyroxin Hormone Level

Introduction

Refereeing has an important part in the dictionary of football game, developing the referees’ abilities and prepare them in a suitable way with the development of the nature of competition between the teams in order to enhance their abilities to make the right decisions that achieve the justice. Thus, the referee and player are the same, both of them needs to the correct preparation in physical, dynamic, knowledge and mental aspects as well as other important personal characteristics such as: leading ability, self-confidence, emotional balance… etc., undoubtedly, refereeing has an essential role in developing football, so when the referees’ level increase that leads to increasing the level of competition. The value of the present research lies in identifying the relationship between refereeing performance, the leading personality and thyroxin hormone level for football referees (First Division), which may be contribute in the performance of referees in a stadium so through these things, referee’s level can be predicted as it achieves high results in these variables.

Practical Framework

The Procedures of the Study

The researchers used the descriptive method of correlation and predictive relationships because of its appropriate to the problem of referees (first division), for the academic year 2018, their number is (15) referees.

Tests:

First: The Measurement of Refereeing Performance:

The multiple forms have been used by the Iraqi Central Iraq Football Association and used in the assessment of the referees of the Iraqi Premier League, which is no different from the referees assessment form.
in the other major leagues and official tournaments. Referee and refereeing team are assessed and sent this form confidentially to the Central Referees Committee where the form contains the level of ease and difficulty of the game with the referee and what mistakes occurred in the game and what the positives possessed by the referee and everything that revolves around the arbitration crew, the results of the test of the referees committee of the Central Iraqi Football Association, which was held on 7-8-9 / 3/2019.

**Second: The Measurement of the Leading Personality:**

The measurement of leading personality has used, which prepared by Al-Janabi, which made up of (47) items, which are answered by choosing one of the two answers for each item. The highest score obtained on the measurement is (47), then the lowest score (zero), i.e. if the measurement degree is high that leads to the presence of adjective.

**Third: The Measurement of Thyroxin Hormone Level:**

The measurement of thyroxin hormone level has done by taking amount of the research samples’ blood, then send to laboratory of Al-Sader Teaching Hospital in order to determine the thyroxin hormone level (T3).

**The Main Experiment:**

The researchers gained the results of the research variables by the following measurements:

1- The results of the refereeing performance were obtained by the Central Iraqi Football Association, the Central Referees Committee for the test which was made in Al-Shaab Stadium on 7-8-9 / 3/2019.

2 - The measurement of leadership personality was distributed between the members of the research sample in the stadium of the College of Physical Education and Sports Science University of Kufa on 15/3/2019, at 10:O’clock in the morning, which took 10 minutes to complete the from.

3 - After completing the process of measuring the leading personality, the researchers took a blood sample from the referees of the research sample through a specialized medical team prepared in advance for this purpose, so the team took a sample of blood and kept it in special tubules and transferred directly to the laboratory for measuring hormone thyroxine level in Al-Sadr Teaching Hospital. Finally, the results were analyzed statistically.

**Statistical Devices:**

The researchers used Statistical Package for Social Sciences (SPSS).

**The Results Discussion**

This framework contained the descriptive statistical results between physical variables and psychological functions after analyzed it in a statistical way and according to the aims of the study.

The Statistical Estimation of the Research Variables for the Research Sample

The statistical estimation of the research variables for the research sample have shown in tables in order to analysis and discussion them, to complete the subsequent statistical analyzes that aims at achieving the aims of the research, so the statistical estimation of the results of the sample studied variables has represented as well as the correlation matrix in which correlations between studied variables have found.

**Table (1): Shows the Statistical Description of Results of the Research Sample in the Studied Variables**

<table>
<thead>
<tr>
<th>No.</th>
<th>Variables</th>
<th>Arithmetic Mean (X)</th>
<th>Standard Deviation (+SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Refereeing Performance</td>
<td>8.24</td>
<td>0.21</td>
</tr>
<tr>
<td>2</td>
<td>Leading Personality</td>
<td>41.56</td>
<td>7.57</td>
</tr>
<tr>
<td>3</td>
<td>Thyroxin Hormone Level</td>
<td>2.54</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Table (1) shows that arithmetic Mean (X) of refereeing performance was (8.24) with standard deviation (0.21), the arithmetic mean (X) of leading personality was (41.56) with standard deviation (7.57) while arithmetic mean (X) of thyroxin hormone level was (2.54) with standard deviation (0.26).
The Correlation Matrix of Relationship Variables

After using Pearson correlation coefficient between the studied variables, the correlation matrix between physical variables and psychological functions has gotten, as shown in table (7):

Table (2): Shows the Correlation Matrix of relationships between the Studied Variables (Physical – Functional)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Refereeing Performance</th>
<th>Leading Personality</th>
<th>Thyroxin Hormone Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refereeing Performance</td>
<td>1</td>
<td>0.547</td>
<td>0.412</td>
</tr>
<tr>
<td>Leading Personality</td>
<td></td>
<td>1</td>
<td>0.438</td>
</tr>
<tr>
<td>Thyroxin Hormone Level</td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

Through the previous table, we can see that there are (45) correlation coefficients, the highest correlation coefficient was (0.547) between refereeing performance and the leading personality i.e. any one of these simple relations between refereeing performance and the leading personality has a significant statistical function... We can find a simple predictive equations.... i.e. to predict the value of refereeing performance by knowing the degree or measurement of leadership personality ... through this we have the opportunity to know the predictive equations complex ... This is a divergent thing that may not serve us at work, so researchers will use the method of preference simple and complex relationships that can be predicted the refereeing performance through any of the studied variables (leadership personality and thyroxin hormone level), as we will see in the following topics. In order to determine the confidence of the correlation coefficients mentioned above, or to know the predictability of a variable and the existence of other variables, the researchers used the alienation coefficient, “an indicator to indicate the characteristics and features that do not share the two variables, so, confidence rate was (5.47%) for The relationship between the refereeing performance and the leading personality, and (4.13%) of the relationship of refereeing performance on the one hand and thyroxin hormone level on the other.

1. Show, Analysis and Discussion the Results of Refereeing Performance and Leading Personality:

The researchers attribute that the refereeing performance has a direct impact on the leading personality, because the presence of the character is the result and an indicator of the refereeing performance that helped the referee, as pointed out by Issam Al-Din Abdel Khaliq Mustafa (that the player have to develop a high degree of physical abilities that are commensurate with the nature of his activity).

Through the previous presentation, the equation of the regression line between the pulse, which obtained the highest correlation with the physical variables as follows:

\[
\text{Refereeing Performance} = 5.904 + (1.848 \times \text{leading personality})
\]

2. Show, Analysis and Discussion the Results of Refereeing Performance and Thyroxin Hormone Level:

Through the previous presentation, the equation of the regression line between refereeing performance that gained the highest coefficient with Thyroxin Hormone, as follows:

\[
\text{Refereeing performance} = 16.243 + (-0.153 \times \text{Thyroxin Hormone})
\]

We can see here that the proportion of public contribution has increased after the participation of the variable (hormone thyroxin) with the 7-11 variable of (leading personality), which in turn is evidence of the impact of variable (refereeing performance) in the proportion of public contribution of the referees and the relationship here positive (i.e. a positive impact) on the level of refereeing performance, through the previous view, the equation of the regression line is as follows:
Refereeing Performance = 10.214 + (0.995× leading personality) + (-0.054× Thyroxin Hormone Level)

Conclusions:
Conclude a final predictive equation through which to predict the refereeing performance in significance of the leading personality and thyroxin hormone level for the referees (First Division). By adoption of the results of the research and utilization by the Football Association to determine the level of referees in significance of the leading personality and thyroxin hormone level of football referees, making researches and similar studies by researchers to study the prediction of the level of refereeing performance in significance of other psychological variables and another sample.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Kufa/ College of Physical Education and Sport Sciences and all experiments were carried out in accordance with approved guidelines.

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Effect of Topical Simvastatin Therapy on Patients with Psoriasis

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Abstract

Objective: Study the effect of simvastatin ointment on tissue expression of inflammatory mediators associated with pathogenesis of Psoriasis such as TNF-α, IL-6 and ICAM-1.

Background: Psoriasis is a common and complex inflammatory disease, which was described as the chronic systemic T-cell-mediated inflammatory condition characterized by the skin and joint manifestation. The patients with psoriasis are usually presented with a white-silvery scaly lesion covering various parts of the body.

In psoriatic lesions, epidermal Keratinocytes produce a variety of inflammatory mediators, such as TNF-α, IL-6, ICAM-1 will stimulate the abnormal proliferation which will cause the psoriasis characteristics to appear.

Simvastatin is an anti-hyperlipidemic agent that acts by competitive, reversible inhibition of the HMG-CoA, the rate-limiting step in cholesterol biosynthesis.

Materials and Method: The study has been carried out on 44 patients suffering from mild to moderate chronic plaque psoriasis; patients with plaque psoriasis will be randomly divided into two groups, the first group was the Placebo group (n 22) whose patients have directed to have placebo plus topical steroid (Dermosalic ointment)® (Betamethasone dipropionate 0.5 mg /g and salicylic acid 30 mg /g) two times for a period of 8 weeks.

The second group was the Simvastatin group (n-22) whose patients have been directed to have Simvastatin ointment 5% two times per day in addition to (Dermosalic ointment)® (Betamethasone dipropionate 0.5 mg /g and salicylic acid 30 mg /g) for a period of 8 weeks. Before and after the eight weeks; The skin biopsy samples have been taken from patients in the two groups at baseline and after eight weeks of treatment used to measure concentrations of TNF-α, IL-6, and ICAM-1.

Conclusion: The present study indicated the fact that Simvastatin ointment plus topical steroid improvement of psoriasis by reduce concentration of inflammatory mediators.

Keywords: Simvastatin ointment, psoriasis, Topical steroid.

Introduction

Psoriasis is a common and complex disease affecting about 2% to 3% of the world’s population. ²⁷ was described as the chronic, systemic T-cell-mediated inflammatory condition associated with skin and joint manifestation. The patients with psoriasis are usually presented with a white-silvery scaly lesion covering various parts of the body. ²⁹. In psoriatic lesions, epidermal Keratinocytes produce a variety of inflammatory mediators, such as cytokine/chemokines ¹⁰. At present, psoriasis is an immunological disorder associated with abnormal keratinocytes proliferation mediated by T-lymphocytes (²⁰, ¹⁵, ²¹). Psoriasis is related to an over expression of pro inflammatory cytokines.
of Th1 cells, and under expression of Th2 cytokines. Now, the effects of cytokines in psoriasis pathogenesis are investigated. However, the biologic activities of cytokines in the in vitro, to the in vivo models in psoriasis, there may be consider as much more complex interactions among individual cytokines in vivo than expected from the in vitro situation\(^\text{18}\). Circulating level of TNF-α is increased in psoriasis disease, and correlated with disease severity\(^\text{5,9}\). TNF-α regulating the ability of antigen-presenting cells (APS) similar to dendrites cells to activate T-cells.\(^\text{28}\)

TNF-α stimulate the expression of C-reactive protein (acute phase response), cytokines such as IL-6 (responsible for T-cell proliferation, and Keratinocyte hyper proliferation); and chemokines such as CCL20 (recruitment of myeloid dendrites cells and T-17 cells) and IL-8 (for recruitment of neutrophils). In the up regulation of intercellular adhesion molecule-I (ICAM-1), TNF-α mediated of the infiltration of inflammatory cells similar to T-cells; and monocytes to the skin. As the role of regulatory T-cells in the pathogenesis of psoriasis remains to be elucidated, IL-6 is thought to render effectors T-cells refractory to regulatory T-cell-mediated suppression.\(^\text{12}\). Plaque psoriasis is considered the commonest type of the disease accounting for 80%–90% of all psoriasis conditions. The psoriatic lesion varies in diameter from one to several centimeters, which may be single or multiple lesions covering almost any part of a body.\(^\text{21}\) The lesions are relatively spread bilaterally symmetrically and most often to be found in the lumbosacral area, knees, scalp, and elbows.\(^\text{8}\).

Topical types of treatment can used for any severity of psoriasis; with limitations based on patient compliance, specific patient needs, and therapy response. It is commonly accepted that a psoriasis-affecting BSA (body surface area) of up to 20% is suitable and is best treated with topical agents using mono therapy.\(^\text{24}\). Topical corticosteroids, the most widely prescribed psoriasis treatment, are effective in the treatment of psoriasis due to their anti-inflammatory, ant mitotic, immunosuppressive and anti-purity properties.\(^\text{17}\).

Inhibitors of HMG-CoA reductase (statins) are commonly used to control blood lipid disorder in medicine. Large clinical studies have shown that patient with or without coronary artery disease, statins significantly decrease cardiovascular morbidity and mortality.

It has also been reported that the use of HMG-CoA reductase inhibitors has immunosuppressive effects. Statins are reductase inhibitors of 3-hydroxy-3-methyl-glutaryl-coenzyme A and besides treating dyslipidemia, pleiotropic anti-inflammatory and immune modulator effects have been found\(^\text{4,7}\).

Statins has been report to cause a reduction in TNF-α, IL-6 , and malondialdehyde (MDA), together with an increased in superoxide dismutase (SOD) act as antioxidant, and cardio protective action.\(^\text{22}\)

Because statins have different pleiotropic effects; new unusual therapeutic modalities for various pathological disorders such as psoriasis; sepsis; alopecia; wound healing, and inflammatory diseases are potentially.

**Patients and methods:**

The present study was conducted from period December-2018 to June-2019. This study was permitted by Kufa university/ College of medicine (Ethical Committee) for clinical trial. Samples were composed from the out patients clinic of Dermatology in ALSaddar teaching hospital clinic.

A total of 44 patients, 25 male, and 19 females were included in this study. patients with plaque psoriasis will be randomly divided into two group:

- **placebo group:** twice daily placebo plus topical steroid (Dermosalic ointment\(^\text{®}\)) were 22 patients (12 patients males; and 10 patients females).
- **Simvastatin group:** Patients treated with (5 % simvastatin ointment) plus topical steroid (Dermosalic ointment\(^\text{®}\)) were 22 patients (13 patients males; 9 patients females).

The libratory study has been conducted in the middle Euphrates cancer unite.
Collection of Samples

Samples of skin biopsy were taken from patients with psoriasis by using a punching tool (5 mm). Five mm of skin were obtained from each subject put into plain disposable tubes contain 10cc normal saline for Elisa was stored at -80˚C in the deep freezer until analysis. Other samples put in formalin for histopathology study.

Homogenization of Samples

Place the tissue sample in eppendorf tube (1.5 cc) by using clean tools, and place it on ice as quick as possibly to prevent degradation with protease. After that add for each 5 mg weight pieces of skin tissue about 300 µL from extraction buffer to eppendorf tube on ice and homogenized manual for about 15 min.

After that Centrifuge by using ice micro centrifuge for 30 min at 14,000 rpm at 4C, when complete take the supernatant by using micropipette to a fresh eppendorf tube and store at -80C.

Statistical Analysis

Data of patients in both studied groups analyzed by using the statistical package version 25, IBM, US, 2017. Descriptive statistics of the variables and studied parameters presented as mean and standard deviation. Gender presented as frequencies and proportions with male to female ratio. Independent two samples students t-test was used to compare the mean difference of a parameter and also used to compare the mean age between both groups. Paired one sample T-test was used to compare the mean of a parameter before and after treatment in each group. Curve estimation, regression analysis was used to assess the correlation between PASI score from one side and each of ICAM-1, IL-6, and TNF-α after treatment from the other side in group 2. R-value which represented the correlation coefficient was calculated. Finally, results were presented in tables and figures with explanatory paragraphs for each using Microsoft Office Word Software for windows version 2013.

Results

Effect of simvastatin on inflammatory mediators

There were not statistically significant difference in baseline of inflammatory mediator values TNF-α ,IL-6 and ICAM-1 between both groups. there were a statistically significant decrease (p˂0.05) in TNF-α ,IL-6 and ICAM-1 after 8 weeks of simvastatin treatment in comparison with baseline in simvastatin treated group, and with that of placebo treated group after 8 weeks .

<table>
<thead>
<tr>
<th>parameter</th>
<th>Placebo group baseline</th>
<th>Placebo group 8weeks</th>
<th>Simvastatin group baseline</th>
<th>Simvastatin group 8weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>15.85(6.14)</td>
<td>4.73(1.88)</td>
<td>12.09(5.21)</td>
<td>7.79(3.81)</td>
</tr>
<tr>
<td>IL-6</td>
<td>77.14(26.54)</td>
<td>29.12(13.10)</td>
<td>52.71(23.18)</td>
<td>9.03(4.22)</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>1.08(0.14)</td>
<td>0.98(0.35)</td>
<td>0.99(0.17)</td>
<td>0.85(0.18)</td>
</tr>
</tbody>
</table>

change of TNF-α, IL-6 and ICAM-1 concentration (pg/ml) of psoriatic patients of the simvastatin and placebo groups. Data expressed as mean ± SD (N=22 in each group) using paired T-test.
The Mean Differences of ICAM-1, IL-6 and TNF level in both groups after treatment:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICAM-1</td>
<td>Placebo</td>
<td>22</td>
<td>0.10</td>
<td>0.03</td>
<td>0.007*</td>
</tr>
<tr>
<td>Pg/ml</td>
<td>Simvastatin</td>
<td>22</td>
<td>0.14</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>Placebo</td>
<td>22</td>
<td>44.97</td>
<td>3.26</td>
<td>0.364*</td>
</tr>
<tr>
<td>Pg/ml</td>
<td>Simvastatin</td>
<td>22</td>
<td>42.10</td>
<td>5.31</td>
<td></td>
</tr>
<tr>
<td>TNF-Alpha</td>
<td>Placebo</td>
<td>22</td>
<td>11.12</td>
<td>3.34</td>
<td>0.001*</td>
</tr>
<tr>
<td>Pg/ml</td>
<td>Simvastatin</td>
<td>22</td>
<td>4.30</td>
<td>2.65</td>
<td></td>
</tr>
</tbody>
</table>

*significant

**Simvastatin group**

<table>
<thead>
<tr>
<th>At baseline</th>
<th>Placebo group</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Baseline Image" /></td>
<td><img src="image2.png" alt="Baseline Image" /></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>After 2weeks</th>
<th>Placebo group</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image3.png" alt="After 2weeks Image" /></td>
<td><img src="image4.png" alt="After 2weeks Image" /></td>
</tr>
</tbody>
</table>
Discussion

Psoriasis is considered a common recurrent Th1-mediated chronic troublesome disease. The recent studies indicated the anti-inflammatory and immunomodulatory effects of statins and used for treatment autoimmune disease, therefore encouraging used of statins for treatment psoriasis.

The current study shows a significant reduction of inflammatory mediator such as TNF-Alpha, IL-6 and ICAM-1 levels in both groups. Compared to baseline concentrations in groups treated with simvastatin and placebo after 8 weeks of therapy, the decline in TNF-Alpha, IL-6 and ICAM-1 concentrations was more significant in simvastatin groups than in placebo group.

who documented a significant decrease in TNF-α and ICAM-1 in psoriatic hyperlipidemic patients after 8 weeks of getting simvastatin orally. to best of our knowledge there is no previous research to compare our result with it. the current study showed that simvastatin could reduce the proliferation, apoptosis, TNF-α, IL-6, and vascular endothelial growth factor secretion both in VSMC and macrophage, which is induced by TNF-alpha activated EC.

reported a significant reduction in IL-6 in patients with acute bacterial infection relative to the control group after receiving simvastatin treatment. this study investigated the relationship between the levels of IL-6, tumor necrosis factor-alpha (TNF-α), and visfatin or simvastatin usage, in the gingival crevicular fluids (GCFs) of diabetic patients with chronic periodontitis; showed the levels reduced due to simvastatin usage.

The study by revealed a significant reduction in plasma ICAM-1 in patients with hyper-lipoproteinemia following simvastatin treatment. Furthermore, reported that in patients with hypercholesterolemia, simvastatin therapy significantly reduced ICAM-1 relative to patients taking bezafibrate for six months.

Conclusion

The present study indicated the fact that Simvastatin ointment plus topical steroid improvement of psoriasis by reduce concentration of inflammatory mediators.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Pharmacology and therapeutics/College of medicine/ University of Kufa, Iraq and all experiments were carried out in accordance with approved guidelines.

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1. Adhwaa S. Effect of oral simvastatin therapy on patients with plaque psoriasis treated with potent topical

Cont.. The Mean Differences of ICAM-1, IL-6 and TNF level in both groups after treatment.

<table>
<thead>
<tr>
<th>After 8 weeks</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
steroid.2019.


5. Arican O, Aral M, Sasmaz S, Ciragil P .Serum levels of TNF-alpha, IFN-gamma, IL-6, IL-8, IL-12, IL-17, and IL-18 in patients with active psoriasis and correlation with disease severity. Mediators Inflamm 2005 ;(5):273–279


Multiplex PCR for detection of the predominant diarrhea-causing protozoa (*Giardia intestinalis*, *Entamoeba histolytica*, and *Cryptosporidium parvum*) in fecal samples of cattle from Babylon Province, Iraq

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Abstract

The work, here, was focused on the detection of the predominant diarrhea-causing protozoa (*Giardia intestinalis*, *Entamoeba histolytica*, and *Cryptosporidium parvum*) in fecal samples of cattle from Babylon province, Iraq. Fifty fecal samples from 50 cattle were collected and processed using a specific multiplex polymerase chain reaction (mPCR) technique that employed the small subunit ribosomal RNA (SSrRNA) gene as a molecular target. Using specific primers for each protozoan, the results showed positive identification of the *E. histolytica*, *G. intestinalis*, and *C. parvum* in 38 (76%), 22 (44%), and 12 (24%), respectively, of the fecal samples. The data from the current investigation identify the presence of the *E. histolytica*, *G. intestinalis*, and *C. parvum* in the fecal samples collected from cattle located in Babylon province, Iraq.

**Keywords:** Cattle, *Cryptosporidium parvum*, *Entamoeba histolytica*, feces, *Giardia intestinalis*, multiplex PCR, protozoa.

Introduction

*Giardia intestinalis*, also known as *Giardia duodenalis*, is a well-recognized protozoan that causes important infections in human and animals. The microorganism was detected in fecal samples of bovine and dairy cattle from various countries of the world (1–5). Clinical diseases caused by *G. intestinalis* in cattle have various symptomatic pictures; however, these could be ranged from no signs appeared on the infected animals to the presence of different types of diarrhea including mucoid and fatty feces, persistent diarrhea, and reduction of weight and growth. The infection of giardiasis can be transmitted to humans through cattle-human direct contact and/or contamination of public water supplies (6–10).

In the case of *E. histolytica*, the protozoan causes amoebiasis which is an important parasitic disease that infects 500 million people with mortalities that can reach up to 40,000 to 100,000 deaths per year. Especially in countries with poor health systems, different infection sources are known for the disease; however, water contaminated with infected human and animal wastes play important roles in spreading the disease (11–16).

Cattle are considered as a major source for infections caused by *C. parvum* inducing a diarrheal disease in humans and loss of production in the infected animals. Also here, contaminated water supplies with feces from infected animals are well-known sources for spreading the infection. 17

Materials and Method

Sample collection

Fifty fecal samples from 50 cattle were collected from multiple fields of cattle in Babylon province, Iraq. The feces were aseptically collected, placed in zip logs, and transferred in an ice box to a laboratory to perform the molecular analyses.

Genomic DNA extraction

The Stool DNA extraction Kit (Bioneer. Korea) was utilized to extract the genomic DNA (gDNA) from the fecal samples. The obtained DNA was read, using a NanoDrop, for identifying its quality and quantity. Then, the DNA was stored at -20°C in a deep freezer.
Polymerase chain reaction

The samples were processed using a specific mPCR technique that employed SSrRNA gene as a molecular target. Using specific primers, table 1, that were designed via the use of NCBI-Genebank and Primer3plus and purchased from (Bioneer Company, Korea).

<table>
<thead>
<tr>
<th>Protozoan</th>
<th>Primer sequence (5’-3’)</th>
<th>Amplicon size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. intestinalis</td>
<td>F CTGGCCCAAGAGTCCTCAAG</td>
<td>488</td>
</tr>
<tr>
<td></td>
<td>R CCGGAGTCGAACCCTGATTCC</td>
<td></td>
</tr>
<tr>
<td>E. histolytica</td>
<td>F ATTGGAGGGCAAGTCTGGTG</td>
<td>389</td>
</tr>
<tr>
<td></td>
<td>R AAATGCTTTCGCTCTCGTGC</td>
<td></td>
</tr>
<tr>
<td>C. parvum</td>
<td>F ATTGGAGGGCAAGTCTGGTG</td>
<td>242</td>
</tr>
<tr>
<td></td>
<td>R CCATGCTGGAGTATTCAAGGC</td>
<td></td>
</tr>
</tbody>
</table>

Then, the AccuPower® PCR PreMix kit (Bioneer, Korea) was employed to prepare the master mix. The tubes of the kit containing a freeze-dried pellet which is the premix components; 1U DNA polymerase, 250µM dNTPs, 10mM Tris-HCl (pH 9.0), 30mM KCl, 1.5mM MgCl₂, a stabilizer, and a tracking dye). Using a total volume of 20µl, the master mix was prepared according to the instructions of the kit and contained DNA template at 5µl, each primer direction at 1.5µl (10pmole), and deionized water to complete the volume up to 20µl. The conditions of the reaction inside a thermocycler (Mygene/Bioneer, Korea) were 1 cycle of 5min at 95°C to perform initial denaturation, 30 cycles of (30s at 95°C to generate denaturation, 30s at 58°C to produce annealing, and 1min at 72°C to perform extension), and 5min at 72°C for final extension. Using an ethidium-bromide-pre-treated 2% agarose gel, the PCR products were electrophoresed and screened using a UV-light based imager.

Results

The results showed positive identification of the E. histolytica, G. intestinalis, and C. parvum in 38 (76%), 22 (44%), and 12 (24%), respectively, of the fecal samples, figure 1, 2, and 3, respectively.

Discussion

Giardia intestinalis is a well-recognized protozoan that causes important infections in human and animals. The current results showed the presence of the protozoan in the fecal samples of cattle in the tested regions of Babylon province, Iraq. These findings agree with (1–5) who detected G. intestinalis in fecal samples of bovine and dairy cattle from various countries of the world. Giardiasis is a zoonotic disease that can be transmitted from different infected animals such as cattle to humans via direct contact with infected cattle and/or their feces and using contaminated public water supplies with this microorganism (9,18–23). The current results match up with those observed by 24 who identified the presence of G. intestinalis in fecal samples of cattle from Urmia, northwest of Iran in a rate of 9.34% using different molecular techniques other than the current one. The differences between the current study detection rate, 44%, could be due to climate differences between Iraq and Iran plus differences between the hygienic systems between the two countries. Moreover, the detection methods could also add variations in the results.

E. histolytica causes amoebiasis which is an important parasitic disease that infects millions of people with thousands of mortalities every year. The current work identified the presence of this protozoan in the fecal samples of cattle from Babylon province, Iraq. Those findings agree with those obtained by (11–16) who identified, especially in countries with poor health systems, different infection sources for the disease occurrence; however, public water contaminated play as important sources in the transmission of the disease.

The present study results showed the identification of C. parvum in the tested fecal samples of cattle from Babylon Province, Iraq, and those findings agree with those recognized by 17 who provided information about the importance of cattle as an infection source
for humans and other animals with inducing a diarrheal disease in humans and loss of production in the infected animals.

Conclusion

The data from the current investigation identify the presence of the *E. histolytica*, *G. intestinalis*, and *C. parvum* in the fecal samples collected from cattle located in Babylon province, Iraq.

Financial Disclosure: There is no financial disclosure.

Conflict of interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Branch of Parasitology, Collage of Veterinary Medicine, University of Al-Qasim Green and all experiments were carried out in accordance with approved guidelines.

References


Study on Breast Cancer Patients and Some Variables in Babylon Province

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Abstract

This study included 100 women with breast cancer in Babylon province for six months at Morjan Teaching Hospital in the Department of Oncology. The study showed that the most age group with breast cancer in the age group of 40 to 49 years, where they accounted for 25% of the proportions of patients. It was also found in the study that the most affected women lived in the center of the city (Urban Area), where they accounted for 60% of the total patient. Most of them were housewives (not working outside the home), with 77%, and 79% of them were first infected with the disease, and 45% of them had a malignant type of tumor.

Keywords: breast cancer, Babylon, age group, pollution

Introduction

Cancer is the result of the growth of cells of the body and spread out of control. Most types of cancer cells are ultimately a mass called tumor, called cancer as the body part of the tumor. Most breast cancers are in the breast tissue of the glands to produce milk, and the cancer is in the ducts that connect the lobes to the nipple. The breast also contains fatty, lymphatic and amniotic tissue. Cancer and death are increasing worldwide, especially in developing countries. One of the most common malignant tumors is breast cancer in all regions of the world and ranks first among cancers affecting women in Iraq. The breast carcinoma is the more repeatedly identified damage and the secondary major source of loss of life in females in Iraq establish 23% of carcinoma associated with end the little duration for the illness is a straight result to the progress produce at recognize. According to the Ministry of Health, the number of cancer cases in 2015 was 25,269. The first breast cancer was 19.1%, the airway and lung (8.1%), leukemia (6.3%), the brain and the central nervous system (CNS) (6.1%), colon and rectum (5.7%), urinary bladder (5.1%) and non-Hodgkin’s lymphoma (4.3%) thyroid (3.8%), skin (3.2%), and stomach (3.2%). Breast cancer was the highest cancer for females (25.8 per 100,000 females). In males, however, the highest rate of lung and lung cancer (6.7 / 100,000 male population).

No qualitative etiological factor has been notarized, but different breast cancer-associated vulnerable factors have been suggested by epidemiological studies; for instance, age, menstrual, menopause, parity, breastfeeding, use of exogenous hormones or oral pill, obesity, lack of exercise, food, smoking, alcohol consumption and family history of breast cancer or other cancers. Exposure to air pollution may also be linked to breast cancer, studies have shown. It has been confirmed that contaminated air is involved in lung cancer and is suspected to be associated with other cancers such as ovaries, brain, breast, bladder and cervix. Breast tumors that have been established in animals by chemical compounds give an indication of the potential impact of environmental pollutants. The data indicate that the cases of new breast cancer, one third of which are known risk factors and many reasons are unknown and the possibility of environmental conditions may also cause breast cancer. The incidence of breast cancer varied to the presence of variables in risk factors. Therefore, the effect of age, place of residence, work and some other variables was studied in this research.

Material and instrument

The study was conducted at the Center of Cancer Oncology of Marjan Educational Hospital of Babylon Health Department where the data were collected by the patients directly. Through the distribution of questionnaire forms to patients coming to the center, which included questions such as (age, place of residence, work, and type of tumor,…etc) The study was conducted during the six months from October 2017 to March 2018.
been working on the number of 100 cases satisfactory.

**Result**

The results showed that breast cancer is common in the age group (40-49) and was 25% of the patients as shown in the figure (1).

![Figure (1) shows the number of infections per age group.](image1)

The results showed that breast cancer had the ability to live in the center of the city (Urban Area) and the lowest percentage was in rural areas as shown in figure (2).

![Figure (2) shows the residential area of breast cancer patients.](image2)

The study showed that most of the patients were not working (housewives) and that the proportion of working women was less as in figure (3).

![Figure (3) shows the proportion of women working and not working (housewives).](image3)

The study also showed that most patients with breast cancer were first infected with 79%, while the remaining 21% were recurrent. The study showed that most of the patients with breast cancer had a malignant tumor of 55% of the infected women, while the remaining 45% had a benign tumor as shown in figure (4).

![Figure (4) shows the type of injury and type of tumor for breast cancer patients.](image4)

**Discussion**

In this study, we found that the age group most affected by breast cancer is the age group of 40 to 49 years Where they accounted for 25% of the total number of patients.

These results are similar to those obtained by Al-Isawi (2016) in Anbar province where most of the patients were The age group of 41-50 got the highest proportion and had an average lifetime of injury 47.16 years old 3 This result is similar to that obtained by the Iraqi Ministry of Health in its report in 2015 that the highest rate of breast cancer was in the age group 45-49 where the number of patients 793 and 40-44 age group was 697 of the total number of 4824 patients for all age groups 5 This result is similar to the results obtained for their study in northern Iraq by Runnak A Majid et al.(2009) With a life expectancy of 47.4 years 11 and This corresponds to what Najjar and Eisson received in their study in 2010 which included 12 Arab countries. The average age of patients was 48 ± 2.8 12. And these results are also close to the study of Molah Karim et al. Which was conducted in 2015 on a group of patients in northern Iraq and their average age was49.4. 13

In this study, the percentage of infection in the center of the city was higher than rural areas where the percentage of infection in the city center was 60% and in rural areas 40%,The high percentage of infection in the center of the city is due to the high pollution in the center of factories, industries and cars compared to the rural area, which enjoys pure air and health and lack of presence of contaminants This result is similar to the report of the Ministry of Health, where the highest incidence was in the center of the city (Hilla) where the proportion of the proportion of 63.2% compared to the rest of the provinces of Babylon of districts and sub-districts (Musayyib, Mahaweel and Hashimiyah) and rural areas. 5 Polluted air has become a major health problem and the problem is growing every day because the inefficient transport system is the main cause of the
air pollution in large cities. The level of air pollution in congested cities is higher than other parts, which can have serious impacts on human health through impact on water and land ecosystems. Beijing, Tehran, Paulo, Sao, Cairo, Shanghai, Cairo, Bangkok, Mexico City and Jakarta are the most polluted cities in the world.

In general, the mortality rate of breast cancer is much greater in the urban population (industrial areas) than the rural population, and the “urban factor” is consistently attributed to the literature of air pollution.

In their studies, researchers found that breast density varied between women living in urban and rural areas. Women living in urban areas have a higher breast density when compared to those living in the rural environment. The cause of high density of non-breast endocrine disorder may result from exposure to air pollutants. Clearly, exposure to air pollutants may be environmental factors that cause breast density because urban and rural areas have different types of air pollution. The researcher Yaghjyan et al. (2017) who conducted a group of women to find out the effect of exposure to air pollution and its relationship to breast cancer, found that the majority of them are from urban areas with 60.3% of those living in rural areas and 39.7% in rural areas.

Many gases and solids are carcinogens and mutants, which in turn lead to many types of cancers. This corresponds to what was obtained by researcher Wei et al. (2012) of a positive relationship between breast cancer incidence and exposure to PAH and NO2, carbon monoxide, sulfur dioxide, volatile organic compounds, micro particles with less diameter about 2.5 microns (PM2.5), and also found Hung et al. (2012) that there was a positive relationship between death from breast cancer and exposure to PM2.5.

The incidence of breast cancer may be due to lack of movement of housewives, lack of exercise and obesity due to unhealthy food. As reported by International agency for research on cancer The main cause of breast cancer is not known. Generally, however, the main causes of breast cancer are not known, but they may not be due to physiological behavior. They may also be due to external factors such as weight differences, reproductive behavior, lack of hormone use, obesity, smoking, fat, and diet. As one of the studies conducted on Iraqi women, most of them do not self-examination of the chest and do not go to the doctor and the reason not to go to the doctor is embarrassment and lack of time and fear of the existence of disease.

### Conclusion

In our study, we concluded that the most age group with breast cancer is 40-49, and that urban areas (city centers) are more affected than rural areas, and working women are less affected than housewives.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Babylon Technical Institute, Al-Furat Al-Awsat Technical University, 51015 Babylon, Iraq and all experiments were carried out in accordance with approved guidelines.

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Knowledge about Obstetric Warning Signs during Pregnancy among Mothers Attending the Primary Health Care Centers in Hilla City

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Abstract

Pregnancy is the most attractive stage in a woman’s life. Signs of risk of pregnancy include bleeding, fluid leakage from the vagina, unusual abdominal pain, cramping, pelvic pressure, headaches or blurred vision, marked swelling in the hands and face, burning urination, foul odors, chills or fever, reduced fetal movements and others with all those the mother need to knowledgeable. A descriptive (cross-sectional) design study was conducted in Al.Hilla city from the period 1st September/2018-13th July/2019, to assess the knowledge toward the obstetric warning signs during pregnancy and to find out the relationship between the knowledge of the study sample and their demographic characteristics with obstetrical history. The sample of the study was non-probability (convenient sample) consist of(250) pregnant women. Data was collected through the use of interview technique, each participant needs(25-30) minutes to complete the form. The questionnaire was validated through a committee of (19) experts. A pilot study was conducted to determine the reliability of the instrument. The data were analyzed using statistical and descriptive data analysis methods carried out by (SPSS version 20). The results of the present study shows that the overall knowledge assessment of the pregnant women were good responses regarding warning signs during pregnancy.

Keywords: Knowledge, Pregnancy, Primary Health Care Centers.

Introduction

Pregnancy is not a disease rather it is a natural life development. However, in many developing countries, warning signs is a major health risk for the mother. Thousands of women and newborns die every year. Almost all the mortality causes related to pregnancy and childbirth are known and most are preventable and treatable. Women with obstetric complications usually die within 48 hours. Complications during pregnancy, such as long-term labor and poor fetal representation, are common causes of perinatal mortality(1).

Danger signs of pregnancies are a warning signs that women encounter during pregnancy, child birth and postpartum. It is important, to know this warning signs for women and health care providers to rule out serious complications and initiate treatment immediately. About 72% of maternal death is attributed to direct causes of maternal mortality(2).

Globally, the maternal mortality was inappropriately high about 830 women die each day due to pregnancy or childbirth problem. In 2015, the normal maternal mortality rate was 216/100,000 live births in developing countries associated with developed countries it was 12/100,000. This high number reproduces inequity in access to health services. The leading effects of maternal deaths were severe vaginal bleeding, infections, pre-eclampsia, eclampsia and unsafe abortion(3).

Knowledge of obstetric warning signs throughout the three steps is an important first step for the applicable organization and timely referral. Increasing knowledge of obstetric warning signs for pregnant women would decrease the delay in seeking care and develop quick detection of obstetric problems(4).

The World Health Organization (WHO) around the world envisages every pregnant woman who may receive a new baby annually for good care during
pregnancy, childbirth and the postpartum. Within primary reproductive health care, antenatal care (ANC) provides a program for important and essential health care functions[5].

In Tanzania demographic health survey report (2011) shows 53% of pregnant women were told about warning signs of pregnancy through ANC visits[6]. In Iraq, the prevalence of maternal death rates around obstetric warning signs, with 84 women dying per 100,000 live births. According to the health sector review and functional review published by the Iraqi Ministry of Health in 2011, Iraq is among a group of 68 countries representing 97% of all maternal and child deaths around the world. While neighboring Iran reduced the maternal mortality rate to 220% between 2000 & 2010, Iraq’s rate declined by one-third during this period[7].

Methodology

A study aims: The purpose of the current study was to assess the knowledge of pregnant women toward the obstetric warning signs during pregnancy and to find out the relationship between the knowledge of the study sample with their demographic characteristics and obstetrical history).

Design of the study: A descriptive (cross-sectional) design study was conducted in Hilla city province from the period 1st September /2018 to 13th July /2019.

Study Sample: The study included nonprobability (convenient sample) consist of (250) pregnant women

Study instrument: A constructed questionnaire was prepared and modified after a thorough review of the relevant literature. This questionnaire covers four parts:

A. Socio-demographical characteristics of the sample includes (age, level of education, occupation, economics status, residency & first pregnancy age)

B. Past obstetric history of the sample includes (gravidity, parity, abortion, gestational age, type of previous delivery, place of delivery & type of antenatal visit)

C. Pregnant women’s knowledge regarding warning signs includes (37) domain

Validity & Reliability: The content validity of the instrument was established through a panel of (19) experts, the reliability of the items was based on the internal consistency of the checklist was assessed by calculating Cronbach Alpha which was = 0.896

A structured questionnaire used to collect data by direct interview. The approximate interview time of 25-30 minutes was provided for the questionnaire completion. Data collection is performed from 18th February - 1st May 2019. To determine whether the objectives of the study were met, the current study data were analyzed by using SPSS, version 25.

Results

Table 1: Socio demographic and Personal Characteristics of the Studied Group (N= 250)

<table>
<thead>
<tr>
<th>Demographic data</th>
<th>Groups</th>
<th>F</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>17-25</td>
<td>88</td>
<td>35.2</td>
</tr>
<tr>
<td></td>
<td>26-34</td>
<td>130</td>
<td>52.0</td>
</tr>
<tr>
<td></td>
<td>&lt;35</td>
<td>32</td>
<td>12.8</td>
</tr>
<tr>
<td>Educational level</td>
<td>Not read&amp;write</td>
<td>8</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>Read&amp;write</td>
<td>51</td>
<td>20.4</td>
</tr>
<tr>
<td></td>
<td>Primary school</td>
<td>37</td>
<td>14.8</td>
</tr>
<tr>
<td></td>
<td>Secondary</td>
<td>79</td>
<td>31.6</td>
</tr>
<tr>
<td></td>
<td>Diploma&amp;above</td>
<td>75</td>
<td>30.0</td>
</tr>
<tr>
<td>Occupation</td>
<td>Unemployed</td>
<td>90</td>
<td>36.0</td>
</tr>
<tr>
<td></td>
<td>Employed</td>
<td>72</td>
<td>28.8</td>
</tr>
<tr>
<td></td>
<td>Student</td>
<td>28</td>
<td>11.2</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>60</td>
<td>24.0</td>
</tr>
<tr>
<td>Socio-Economic status</td>
<td>Satisfied</td>
<td>135</td>
<td>54.0</td>
</tr>
<tr>
<td></td>
<td>Satisfied to some extent</td>
<td>90</td>
<td>36.0</td>
</tr>
<tr>
<td></td>
<td>Unsatisfied</td>
<td>25</td>
<td>10.0</td>
</tr>
<tr>
<td>Residency</td>
<td>Urban</td>
<td>176</td>
<td>70.4</td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>74</td>
<td>29.6</td>
</tr>
<tr>
<td>First pregnancy age</td>
<td>&lt;20</td>
<td>83</td>
<td>33.2</td>
</tr>
<tr>
<td></td>
<td>20-29</td>
<td>137</td>
<td>54.8</td>
</tr>
<tr>
<td></td>
<td>≥ 30</td>
<td>30</td>
<td>12.0</td>
</tr>
</tbody>
</table>
Table (1) The highest percentage of pregnant women (52.0%) was found to be (26-34) years (31.6%), and the profession of mothers in this sample (36.0%) of housewives (54.0%) and their economic situation was sufficient. In terms of accommodation, (70.4%) of participants live in urban areas, and the first pregnancy age were (20-29) (54.8%).

Table 2. Past Obstetric History of the Study Sample (N=250)

<table>
<thead>
<tr>
<th>Past obstetric history</th>
<th>Groups</th>
<th>F</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parity</td>
<td>Primi-para</td>
<td>59</td>
<td>23.6</td>
</tr>
<tr>
<td></td>
<td>Multi-para</td>
<td>191</td>
<td>76.4</td>
</tr>
<tr>
<td>Gravidity</td>
<td>Once</td>
<td>52</td>
<td>20.8</td>
</tr>
<tr>
<td></td>
<td>Twice</td>
<td>73</td>
<td>29.2</td>
</tr>
<tr>
<td></td>
<td>3&amp;more</td>
<td>125</td>
<td>50.0</td>
</tr>
<tr>
<td>Abortion</td>
<td>None</td>
<td>145</td>
<td>58.0</td>
</tr>
<tr>
<td></td>
<td>Once</td>
<td>93</td>
<td>37.2</td>
</tr>
<tr>
<td></td>
<td>&gt;1</td>
<td>12</td>
<td>4.8</td>
</tr>
<tr>
<td>Gestational age</td>
<td>1–11 weeks</td>
<td>48</td>
<td>19.2</td>
</tr>
<tr>
<td></td>
<td>12–27 weeks</td>
<td>124</td>
<td>49.6</td>
</tr>
<tr>
<td></td>
<td>28–40 weeks</td>
<td>78</td>
<td>31.2</td>
</tr>
<tr>
<td>Type of previous delivery</td>
<td>Normal delivery</td>
<td>146</td>
<td>58.4</td>
</tr>
<tr>
<td></td>
<td>Cesarean/section</td>
<td>48</td>
<td>19.2</td>
</tr>
<tr>
<td></td>
<td>Primi-gravide</td>
<td>56</td>
<td>22.4</td>
</tr>
<tr>
<td>Place of previous delivery</td>
<td>Home</td>
<td>39</td>
<td>15.6</td>
</tr>
<tr>
<td></td>
<td>Traditional Birth Attendance (TBA)</td>
<td>20</td>
<td>8.0</td>
</tr>
<tr>
<td></td>
<td>Hospital</td>
<td>135</td>
<td>54.0</td>
</tr>
<tr>
<td></td>
<td>Primi-gravide</td>
<td>56</td>
<td>22.4</td>
</tr>
<tr>
<td>Type of antenatal visit</td>
<td>Regular</td>
<td>144</td>
<td>57.6</td>
</tr>
<tr>
<td></td>
<td>Irregular</td>
<td>106</td>
<td>42.4</td>
</tr>
</tbody>
</table>

Table (2) indicates that the highest percent of the sample regarding parity (76.4%), had the highest number of gravity (50%), without any abortion (58.0%), and most pregnant mothers in the second trimester (49.6%), (58.4%) was normal vaginal delivery. In the hospital (54.0%) and visits to health centers were regular (57.6%).

Table (3) Overall of Mothers Knowledge Scores toward Obstetric Warning Signs during Pregnancy

<table>
<thead>
<tr>
<th>Overall knowledge</th>
<th>Responses</th>
<th>F</th>
<th>%</th>
<th>Mean</th>
<th>S.D</th>
<th>Ass.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Don’t know</td>
<td>10</td>
<td>4</td>
<td></td>
<td>2.60</td>
<td>.459</td>
<td>Good</td>
</tr>
<tr>
<td>Not sure</td>
<td>75</td>
<td>30.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I know</td>
<td>165</td>
<td>66</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>250</td>
<td>100.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (3) demonstrates that the overall responses of pregnant women are good regarding knowledge.
Table(4) Relationship between the Mothers Knowledge toward Warning Signs during Pregnancy & their Demographic Data

<table>
<thead>
<tr>
<th>Demographic data</th>
<th>Groups</th>
<th>Overall assessment</th>
<th>(\chi^2)</th>
<th>d.f</th>
<th>P. value</th>
<th>sig</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Don’t know</td>
<td>Not sure</td>
<td>I know</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>17-25</td>
<td>6</td>
<td>41</td>
<td>41</td>
<td>18.768</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>26-34</td>
<td>4</td>
<td>25</td>
<td>101</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&lt;35</td>
<td>0</td>
<td>9</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Educational level</td>
<td>Not read&amp;write</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>.940</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Read&amp;write</td>
<td>6</td>
<td>15</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Primary school</td>
<td>1</td>
<td>11</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Secondary</td>
<td>0</td>
<td>26</td>
<td>53</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diploma &amp;above</td>
<td>0</td>
<td>20</td>
<td>55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td>Unemployed</td>
<td>6</td>
<td>23</td>
<td>61</td>
<td>5.540a</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Employed</td>
<td>1</td>
<td>24</td>
<td>47</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Student</td>
<td>0</td>
<td>13</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>3</td>
<td>15</td>
<td>42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Socio-economic status</td>
<td>Satisfied</td>
<td>2</td>
<td>38</td>
<td>95</td>
<td>1.496a</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Satisfied to some extent</td>
<td>3</td>
<td>31</td>
<td>56</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unsatisfied</td>
<td>5</td>
<td>6</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residency</td>
<td>Urban</td>
<td>8</td>
<td>57</td>
<td>111</td>
<td>1.612a</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Rural</td>
<td>2</td>
<td>18</td>
<td>54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First pregnancy age</td>
<td>&lt;20</td>
<td>3</td>
<td>40</td>
<td>40</td>
<td>21.007a</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>20-29</td>
<td>1</td>
<td>26</td>
<td>110</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥ 30</td>
<td>6</td>
<td>9</td>
<td>15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table(4) indicates that there were highly significant relationship between pregnant women’s knowledge with their demographical data regarding age & first pregnancy age, while the study result shows that there was no-significant relationship with other demographical data such as educational level, occupation, socio-economic & residency.
Table(5) Relationship between the Mothers Knowledge about Warning Signs during Pregnancy and their Past Obstetric History(N=250)

<table>
<thead>
<tr>
<th>Past obstetric</th>
<th>Groups</th>
<th>Overall assessment</th>
<th>(\chi^2)</th>
<th>d.f</th>
<th>P. value</th>
<th>sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parity</td>
<td>Primi-para</td>
<td>Don’t know 24</td>
<td>4.193a</td>
<td>1</td>
<td>.041</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>Multi-para</td>
<td>Not sure 51</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>I know 135</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gravidity</td>
<td>Once</td>
<td>Don’t know 5</td>
<td>2.212a</td>
<td>2</td>
<td>.331</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Twice</td>
<td>Not sure 17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3&amp;more</td>
<td>I know 82</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abortion</td>
<td>None</td>
<td>Don’t know 8</td>
<td>10.289a</td>
<td>2</td>
<td>.006</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>Once</td>
<td>Not sure 39</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;1</td>
<td>I know 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age</td>
<td>1–11 weeks</td>
<td>Don’t know 7</td>
<td>3.628a</td>
<td>2</td>
<td>.163</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>12–27 weeks</td>
<td>Not sure 44</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>28–40 weeks</td>
<td>I know 58</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of previous delivery</td>
<td>Primi-gravida</td>
<td>Don’t know 4</td>
<td>5.193</td>
<td>2</td>
<td>.075</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Normal delivery</td>
<td>Not sure 44</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cesarean/section</td>
<td>I know 9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Place of previous delivery</td>
<td>Primi-gravida</td>
<td>Don’t know 4</td>
<td>5.193</td>
<td>2</td>
<td>.075</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Home</td>
<td>Not sure 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Traditional birth attendance</td>
<td>I know 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hospital</td>
<td>Not sure 42</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of antenatal visit</td>
<td>Regular</td>
<td>Don’t know 5</td>
<td>1.376a</td>
<td>1</td>
<td>.241</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Irregular</td>
<td>Not sure 36</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table(5) shows that there was highly significant relationship between pregnant women’s knowledge with their past obstetric history at(P>0.05) regarding to parity & abortion, while the study result shows that there was the no-significant relationship between other past obstetric history such as gravidity, gestational age, type of previous delivery, place of previous delivery and type of antenatal visit.
Discussion

- Socio-Demographic characteristic

The results of the study showed that most of the mothers were in the age group (25-34) years, most of females in this age may get an opportunity to marry and can give birth. In a cross-sectional study of (359) pregnant women, it was reported by (7) that majority of mothers’ ages were (26-34) years.

In terms of educational level sample was distributed between secondary graduates, diploma and above. Mostly in Iraq and because some of cultural and other issues women may have only secondary education. And this is disagreed with (8) who reported that the sample educational level in their study was (79%) with primary education.

More than 50 per cent of the sample were satisfied with their economic situation from their point of view and according to their needs and their families requirement. While (9) found that economic situation of most Bangladesh mothers was unsatisfied.

The current study results illustrated that high percentage of sample were mothers live in urban areas, this can be justified due to the selection of the study sample as well as to the system followed by the Ministry of health and their distribution of the primary health care centers. This is agreed with (10) who stated that majority of participants about three fourth (76.7%) were urban residents.

Concerning the age of first pregnancy most mothers represented (54.8%) had their first one between the ages of (20–29). Most of the literatures verified that this is the reasonable age for pregnancy. (11) stated that (80.1%) became pregnant before 25 years of age.

- Past Obstetric History of the Study

The results of the study showed the high percentage of the sample was found multipara, multigravida, without any history abortion, and most pregnant mothers were in second trimester. Most of this study sample attending the health center for different reasons. Many researchers study, the obstetrical history as variable because it is related much to the topic of maternal health. (3) stated that among (137) had more than four children. About three-fourths of participants (75.9%) did not have any previous abortion.

The present study found that more than half of the participants had normal delivery, the same table illustrated that (54%) of the mothers delivered in the hospital and more than half of the sample had no risk before pregnancy (59.2%). In cross-sectional study which was conducted by (12) of 300 of pregnant women in Libya found in the history of the previous pregnancy that, majority of the pregnant women had a normal pregnancy. 71.4% of the multiparous women mentioned that they had previous normal vaginal deliveries.

The same results indicated that (57.6%) of the sample their visits to health centers were regular. A descriptive cross-sectional study was done among 170 participants conducted by (13) in India.

Overall Mothers Knowledge about Warning Signs

The result of the current study showed that most of mothers had (70%) good knowledge toward obstetric warning signs. More supportive evidence is provided by (14) in Indian who carried out a cross-sectional survey on mothers about “knowledge of danger signs of pregnancy, labor and post-partum period among mothers in rural Pondicherry.

Relationship between Mothers Knowledge & Demographic Characteristics

Socio-demographic factors can affect women’s knowledge related risk signs during pregnancy. The results showed that age was significantly associated with the level of knowledge of women. Similar findings have been reported the woman in studies from Malaysia (15). The best knowledge they have besides their previous pregnancy and childbirth experiences, especially for those who have had complications of childbirth during their previous pregnancy (16). The occupational status seems to have an impact on women’s knowledge. The study which done in Punjab by (17) matched the present results.

While the level of education appears to play a positive role in increasing women’s knowledge of signs of risk during pregnancy. Educated women have better access to reproductive health information than uneducated women, are more independent in decision making and have greater access to quality health care services. The results of this study are inconsistent with a cross-sectional study by (18) in Maharashtra, who found that there was a statistically significant relationship...
between the level of education & the mother's knowledge

**Relationship between the Mothers Knowledge & Past Obstetric History**

The result of the presented study shown significant relationship between pregnant women’s knowledge with past obstetric history (parity & abortion) this results agreement with a study done in Baghdad conducted by [19], found that parity, abortion, statistically significant with the knowledge of pregnant

While show that the non-significant relationship between regarding gravid & else, this results agreement with a study done in Debre Birhan [10].

**Conclusions**

Most of the pregnancy who attended the PHC centers are young age multipara in their second trimester of gestation and their visits were regular. Most of mothers have heard information from family, relative and friends. The overall assessment of knowledge about warning signs during pregnancy was good.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Community health Department and all experiments were carried out in accordance with approved guidelines.

**References**

6. Webster PC. Roots of Iraq’s maternal and child health crisis run deep. The Lancet, 2013; 381(9870): 891-894


EEG Changes in Neonatal Hypoxic Ischemic Encephalopathy

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Abstract

Background: Traditional multichannel EEG is an integral part of the evaluation of neonates diagnosed with neonatal hypoxic ischemic encephalopathy (HIE). HIE is well-defined as the clinical appearance of reduced neonatal brain function after asphyxia due to perinatal and/or an antenatal adverse incident. It is prevalence ranging from 1 to 8 per 1000 worldwide. Objectives: To evaluate EEG changes in neonates with hypoxic ischemic encephalopathy.

Method: A cross sectional study conducted from Aug. 2018 till June 2019. The patient included ,term neonate with gestational age from (37 to 42 weeks) admitted in Neonatal Care Unit(NCU) in Babylon teaching hospital for maternity and children , with diagnosis of hypoxic Ischemic Encephalopathy(HIE ). with total number of 47 were enrolled in the study. Full assessment, including: clinical assessment (history and full examination) and electrophysiological (EEG) were done to all Patients. First EEG was done at age ranged from (2 to 7 days) while second EEG was done at age ranged from (21 to 28 days).

Result: In this study, first EEG showed that 8 (17.02%) normal EEG and 39 (82.9%) abnormal EEG results, while second EEG was shown that 15 (31.9%) normal EEG, 17 patients get improvement and 7 get worse. This study was shown that, there are a strong significant statistical deference between the EEG background activity in the first week and developmental mile stone, stage of HIE, neurological examination, age of patients and seizure with p value less than 0.05.

Conclusion: EEG of neonates with HIE provides early prognostic and objective information.

Key words: EEG, neonatal hypoxic ischemic encephalopathy

Introduction

HIE has significant detrimental effects on the growing brain and is between the leading causes of death among neonates, as well as the major original cause of seizures in term neonates ¹. HIE is still a serious situation that is unresolved and causes significant mortality and long-term morbidity ². Neonatal HIE can also be considered as damage that occurs in the immature brain, producing in delayed cell death via excitotoxicity, oxidative stress and inflammation. These adverse happenings in the evolving brain often lead to long lasting detrimental neurological defects afterward in life such as mental retardation, learning disabilities, epilepsy, cerebral palsy, and other neurophysiological handicaps ³.

The “EEG picture” of a disease is frequently a visual waveform or an atypical frequency or abnormalities in waveform amplitude or a hyper synchrony ⁴. The EEG in neonates with HIE reveals the severity of brain damage and changes over time ⁵.

Method

Inclusion criteria:

1-Term (37-42 weeks of gestation).
2-HIE occur in neonatal period 28 days.
3- fitting within 7 days of delivery.

The patients were selected according to the American Academy of Pediatrics and American College of Obstetrics and Gynecology ⁶ criteria, a patient with
hypoxic ischemic encephalopathy require 3 or more of the following criteria.

(a) Neonatal encephalopathy.
(b) Apgar score ≤ 3 for elongated than 5 minutes.
(c) Intense metabolic acidosis (pH < 7.0) in umbilical artery blood.
(d) Multiorgan system failure.
(e) Fetal bradycardia. Patients in this study have low Apgar score, neonatal encephalopathy and fetal bradycardia

Exclusion criteria:
1- Infant with cerebral Sino venous thrombosis.
2- Newborns with other structural CNS abnormalities.
3- Encephalopathy due to genetic disorders, infections and inborn error of metabolism.

Table 1 Classification of EEG Background Activity

<table>
<thead>
<tr>
<th>Grade</th>
<th>Results</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal EEG</td>
<td>EEG results Continuous background arrangement with normal physiologic features for example anterior slow waves.</td>
</tr>
<tr>
<td>1</td>
<td>Normal/mild</td>
<td>Continuous background arrangement with slightly abnormal activity (slight asymmetry, slight voltage depression).</td>
</tr>
<tr>
<td></td>
<td>abnormalities</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Moderate abnormalities</td>
<td>Discontinuous activity by way of interburst interval of &lt;10 s, or clear asynphony or asymmetry.</td>
</tr>
<tr>
<td>3</td>
<td>Major abnormalities</td>
<td>Discontinuous activity with interburst interval of 10–60 s, marked Reduction of background patterns.</td>
</tr>
<tr>
<td>4</td>
<td>Inactive EEG findings</td>
<td>Background action of &lt;10 μV or severe discontinuity with IBI of &gt;60 s</td>
</tr>
</tbody>
</table>

EEG Procedure

An EEG was done in department of neurophysiology in the hospital of Imam AL Sadiq. A neonate was lied on a bed and the EEG electrodes were located to the scalp at FP1, FP2, F3, F4, C3, C4, P3, P4, F7, F8, T5, T6, T3, T4, O1, O2, and CZ permitting to the international 10-20 system of electrode placement using adhesive paste. A pair of electrodes is required in order to get a voltage potential difference. Most EEGs was taken about 1 hour. Before the procedure, the patient’s parents are requested to wash their child hair the night before the test and stop taking certain medications before the test. The parents of neonates satisfying the criteria were approached and informed consent was achieved as soon as after the birth or with the start of clinical seizures. All EEGs were given a grade depended on background activity, which has been described Table below.
Results

The patient’s age ranged from 2 – 7 days and most of them are male and have birth weight more than 3.75 kg shown in table below.

Table 2 Distribution of the study patients according to child characteristics.

<table>
<thead>
<tr>
<th>variables</th>
<th>Mean</th>
<th>percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Mean</td>
<td>5.45 (±1.47)</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>31 (66%)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>16 (34%)</td>
</tr>
<tr>
<td>Gestational age</td>
<td>Mean</td>
<td>38.6 (± 1.33)</td>
</tr>
<tr>
<td>Birth weight</td>
<td>Less than 2.5 kg</td>
<td>7 (14.9%)</td>
</tr>
<tr>
<td></td>
<td>2.5-3.750 kg</td>
<td>19 (40.4%)</td>
</tr>
<tr>
<td></td>
<td>More than 3.750kg</td>
<td>21 (44.7%)</td>
</tr>
<tr>
<td>Mode of delivery</td>
<td>Home delivery</td>
<td>3 (6.4%)</td>
</tr>
<tr>
<td></td>
<td>Hospital vaginal delivery</td>
<td>34 (72.3%)</td>
</tr>
<tr>
<td></td>
<td>Cesarean section</td>
<td>10 (21.3%)</td>
</tr>
</tbody>
</table>

This study was shown that most of patients have moderate HIE scale 48.9%, the staging was done according to modified sarant scale and have normal development mile stone 66% (patients were followed up from age ranged from 3-11 months).

Table 3 Distribution of the study group according to the neurological characteristics

<table>
<thead>
<tr>
<th>characteristics</th>
<th>group</th>
<th>NO. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIE</td>
<td>Mild</td>
<td>20 (42.6%)</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>23 (48.9%)</td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>4 (8.5%)</td>
</tr>
<tr>
<td>Neurological examination</td>
<td>Normal</td>
<td>13 (27.7%)</td>
</tr>
<tr>
<td></td>
<td>Hypotonic</td>
<td>23 (48.9%)</td>
</tr>
<tr>
<td></td>
<td>Spastic (hypertonia)</td>
<td>11 (23.4%)</td>
</tr>
<tr>
<td>Developmental milestones</td>
<td>Normal</td>
<td>31 (66%)</td>
</tr>
<tr>
<td></td>
<td>delayed</td>
<td>16 (34%)</td>
</tr>
<tr>
<td>Presence of Seizure</td>
<td>Yes</td>
<td>23 (49%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>24 (51%)</td>
</tr>
</tbody>
</table>
Table 4 Distribution of study patients in the first and second EEG.

<table>
<thead>
<tr>
<th>EEG Type</th>
<th>1st week</th>
<th>4th week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>normal</td>
<td>8</td>
<td>17.0</td>
</tr>
<tr>
<td>mild</td>
<td>13</td>
<td>27.7</td>
</tr>
<tr>
<td>moderate</td>
<td>12</td>
<td>25.5</td>
</tr>
<tr>
<td>major</td>
<td>12</td>
<td>25.5</td>
</tr>
<tr>
<td>inactive</td>
<td>2</td>
<td>4.3</td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td>100.0</td>
</tr>
</tbody>
</table>

In this study there are positive association between EEG background activity in the 1st week and age of patient p value less than 0.05 while there are no significant association between EEG background activity in the 1st week and gender, mode of delivery, birth weight and gestational age.

Table 5 The association between different child characteristics and EEG findings in the 1st week

<table>
<thead>
<tr>
<th>normal</th>
<th>abnormal</th>
<th>abnormality of EEG</th>
<th>Total</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of the baby</td>
<td></td>
<td></td>
<td></td>
<td>0.011</td>
</tr>
<tr>
<td>2-7 days</td>
<td>No.</td>
<td>8</td>
<td>39</td>
<td>47</td>
</tr>
<tr>
<td>%</td>
<td>17.0%</td>
<td>82.9%</td>
<td>100.0%</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td>0.963</td>
</tr>
<tr>
<td>Male</td>
<td>Count</td>
<td>6</td>
<td>25</td>
<td>31</td>
</tr>
<tr>
<td>% within gender2</td>
<td>19.4%</td>
<td>80.6%</td>
<td>100.0%</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>Count</td>
<td>2</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>% within gender2</td>
<td>12.5%</td>
<td>87.5%</td>
<td>100.0%</td>
<td></td>
</tr>
<tr>
<td>Birth weight</td>
<td></td>
<td></td>
<td></td>
<td>0.451</td>
</tr>
<tr>
<td>Less than 2.5 kg</td>
<td>Count</td>
<td>0</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>% within Wt2</td>
<td>.0%</td>
<td>100.0%</td>
<td>100.0%</td>
<td></td>
</tr>
<tr>
<td>2.5 to 3.750 kg</td>
<td>Count</td>
<td>3</td>
<td>16</td>
<td>19</td>
</tr>
<tr>
<td>% within Wt2</td>
<td>15.8%</td>
<td>84.2%</td>
<td>100.0%</td>
<td></td>
</tr>
<tr>
<td>More than 3.750 kg</td>
<td>Count</td>
<td>5</td>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td>% within Wt2</td>
<td>23.8%</td>
<td>76.2%</td>
<td>100.0%</td>
<td></td>
</tr>
<tr>
<td>Gestational age</td>
<td></td>
<td></td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td>less than 39 wks</td>
<td>Count</td>
<td>4</td>
<td>19</td>
<td>23</td>
</tr>
<tr>
<td>% within G.age2</td>
<td>17.4%</td>
<td>82.6%</td>
<td>100.0%</td>
<td></td>
</tr>
<tr>
<td>39-40 wks</td>
<td>Count</td>
<td>3</td>
<td>16</td>
<td>19</td>
</tr>
<tr>
<td>% within G.age2</td>
<td>15.8%</td>
<td>84.2%</td>
<td>100.0%</td>
<td></td>
</tr>
<tr>
<td>41 or more</td>
<td>Count</td>
<td>1</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>% within G.age2</td>
<td>20.0%</td>
<td>80.0%</td>
<td>100.0%</td>
<td></td>
</tr>
<tr>
<td>Mode of Delivery</td>
<td></td>
<td></td>
<td></td>
<td>0.808</td>
</tr>
<tr>
<td>Home delivery</td>
<td>Count</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>%</td>
<td>.0%</td>
<td>100.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospital Vaginal Delivery</td>
<td>Count</td>
<td>7</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>%</td>
<td>20.6%</td>
<td>79.4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cesarean section</td>
<td>Count</td>
<td>1</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>%</td>
<td>10.0%</td>
<td>90.0%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
This study was shown that, there are a strong positive association between the EEG background activity in the first week and development milestone, stage of HIE, neurological examination and seizure with p value 0.0001.

**Table 6 The association between neurological characteristics and the EEG findings in the 1st week.**

<table>
<thead>
<tr>
<th>Development milestone</th>
<th>EEG background activity in the 1st week</th>
<th></th>
<th></th>
<th>0.0001*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>No. 8 13 8 2 0 31</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>% 25.8% 41.9% 25.8% 6.5% .0% 100.0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delay</td>
<td>No. 0 0 4 10 2 16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>% .0% .0% 25.0% 62.5% 12.5% 100.0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seizure</td>
<td>No. 8 10 5 0 1 24</td>
<td></td>
<td></td>
<td>0.0001*</td>
</tr>
<tr>
<td></td>
<td>% 33.3% 41.7% 20.8% .0% 4.2% 100.0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>No. 0 3 7 12 1 23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>% .0% 13.0% 30.4% 52.2% 4.3% 100.0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage of HIE</td>
<td>No. 8 9 3 0 0 20</td>
<td></td>
<td></td>
<td>0.0001*</td>
</tr>
<tr>
<td>Mild</td>
<td>% 40.0% 45.0% 15.0% .0% .0% 100.0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>No. 0 4 8 9 2 23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>% .0% 17.4% 34.8% 39.1% 8.7% 100.0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>No. 0 0 1 3 0 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>% .0% .0% 25.0% 75.0% .0% 100.0%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Measured by fissure exact test

In this study, 2nd EEG finding shows that there are a significant association in a group not take AEDs with p value 0.021

**Table 7 Significance of difference in EEG Findings after 4 wks. (By wilcoxin rank- signed test).**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group</th>
<th>N</th>
<th>Mean rank</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both groups</td>
<td>Negative rank</td>
<td>17</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive rank</td>
<td>7</td>
<td>13.71</td>
<td>0.083</td>
</tr>
<tr>
<td></td>
<td>No change</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated group</td>
<td>Negative rank</td>
<td>7</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive rank</td>
<td>5</td>
<td>7.2</td>
<td>0.796</td>
</tr>
<tr>
<td></td>
<td>No change</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Table 7 Significance of difference in EEG Findings after 4 wks. (By willcoxin rank- signed test).**

<table>
<thead>
<tr>
<th>Untreated group</th>
<th>Negative rank</th>
<th>10</th>
<th>6.5</th>
<th>0.021**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive rank</td>
<td>2</td>
<td>6.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No change</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Discussion** In our study first EEG shows that 82.9% patients have abnormal EEG and 17% with normal EEG while in the second EEG the numbers of normal EEG became 31.9% and from those with abnormal first EEG 17.9% patients return to normal in second EEG. This mean most of the cases, EEG grades either remained constant or improved between first and second EEG this result agree with 8 in this study was used the same EEG feature we depend on for a classification of EEG background activity (Continuity, asymmetry, voltage depression and interburst interval or asynchrony). 9 tackled 31 infants and do EEGs within first week of infant age and founded that 17 normal EEG from them 15 still normal while 2 became abnormal, 13 BS all stayed abnormal. 7 founded that The EEG grade allocated correlated considerably with outcome and EEG abnormalities improved with time, with the worst EEG grade seen on the initial recording in all cases. A substantial problem in the classification of different abnormal EEG features is that there are no universal definition of diverse abnormalities in terms of voltage level, phase or frequency. For instance, voltage levels used to define an abnormality such as low voltage often differ among studies.

In this study there are a significant association between EEG background activity in the 1st week and age of patient with p value 0.011 and there are no significant association with others child characteristics. 10 has similar result to our study while (11,8) show disagreement with our study in that they found there are positive association with mode of delivery. 12 reported high incidence of birth asphyxia in rural area this result shown agreement with our study since 3 patients with home delivery all have abnormal EEG finding, 13 found that home delivery associated with more severe birth asphyxia and abnormal neurodevelopment outcome.

According to development mile stone we found that patients were classified as having mild EEG feature all with normal development which has agreement with 14, also 15 was shown that, infants with mild HIE with a continuous or to some extent discontinuous a EEG with lacking cycllicity are more likely to have the good outcome, while infants with severe HIE have a severely abnormal a EEG background (low voltage, inactive) have a high threat for adverse outcome (death or severe handicap) this result show agreement with our study since patients with severe EEG grade (10 from 12 patients with major EEG grade and all 2 patients with inactive EEG grade) associated with delay development. Explanation to these result are background features of EEG accurately expect long term neurodevelopmental outcome in term neonates with HIE. Burst suppression, flat trace and low voltage do predict neurodevelopmental outcome with a great specificity and sensitivity 14.

Patients with HIE stage one distributed between normal, mild and moderate EEG grades most of them have mild EEG grade 45% while patients with moderate HIE stage two distributed in all EEG grades with most of them have major 39.1% and the patients with severe HIE stage three distributed between moderate 25% and major 75% EEG grade 11 show agreement with our study by finding that, there are positive association between EEG background feature and severity of HIE, While 18 shown disagreement to our study in that all neonates were on hypoxic-ischemic encephalopathy (HIE) Stage I had normal EEG recording; 36.7% with HIE Stage II had abnormal EEG recording while had agreement to our study in that 100% of the neonates with HIE Stage III showed abnormal EEG. Explanation to this, the severity of encephalopathy in asphyxiated neonates correlates well with the abnormalities on EEG records.

**Conclusion**

EEG regard as the safest mode for a neonate with convulsion and informative tool for routine work up for HIE patient even in neonate without fitting.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the University of Babylon /
Babylon Medical College, Iraq and all experiments were carried out in accordance with approved guidelines.

References


Knowledge Attitudes and Barriers of Undergraduate Medical Students towards Research in University of Babylon

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1 Lecturer, 2 Prof. Dr., 3Assist. Prof., University of Babylon – Hammurabi Medical College, Iraq

Abstract

Students who did not participate in this activity during their training that might negatively affect their understanding of the importance of research in their future careers.

Objective of the study: To evaluate the attitude and knowledge and barriers of a sample of Iraqi medical undergraduate students.

Methodology: Across sectional study was done using a self-administered pretested questionnaire to measure the knowledge, barriers and attitudes of students toward research.

Results: Two hundred eight medical students were studied in the University of Babylon. The female to male ratio was 2:1. Regarding barriers, the study revealed that (55.3%) of the students had efficient internet. This study explained that the skill of English language writing is very low. Only one-fourth of the students were exposed to the encouragement to participate in academic research and 8.7% of the students mentioned that there was available chance for them to conduct research. Only 15.8% agreed that they can access scientific journals through university library while 3.4% of study group knew that there are government financial support toward research, 68.2% of the students said that there are no enough training in research. Regarding the attitude 73.3% of the participants had positive attitudes. The high majority of the students (85%) believed that research is highly important for their medical practice.

Keywords: medical students, undergraduate, knowledge, attitude, barriers, Babylon.

Introduction

Research is a systematic process and simply research can be defined as “a systematic collection of data that uses disciplined methods (1,2). Research during undergraduate study is essential to reform health system this activities help in addressing the root of health problems and mitigating their risk factors (3). Research is a paramount importance for application of all levels of prevention and promotion of health program (4). Medical students usually are very busy during their study to engage in conducting medical scientific research. This is more common in source limited developing countries (5). It is well known that of undergraduate medical students involvement in research performance will help them to be a good researcher after completing their training (6). However, students are too busy to find sufficient time for research activities (7). Modern medical school’s curriculum include record programs (8). Training undergrad students on record provide students with transferable skills of communications, improve managerial skills, knowledge positive attitudes and practices in critical appraisal skills programs and practicing evidence base. Medical student involvement in research has been declining over the years (9). Medical students’ engagement in research has been associated with the acquisition of teamwork, fostering positive attitudes toward scientific methodology (10,11). Unfortunately, recent reports point to declining numbers of clinician–scientists in both developed and developing countries (12,13). In conducting research inadequate knowledge is one of the most common reasons behind suboptimal study design or interpretation (14,15). The syllabus of the education curriculum should be changed to deal with lack of training in research methodology using small group learning approach (16). The factors that affect the research training are the knowledge, the attitudes and the barriers (17). This study was done to evaluate the knowledge, attitude to and the barriers...
toward research among undergraduate medical and dentistry students.

**Methodology**

The acceptance of the ethical committee of scientific researches in Hammurabi college of medicine was taken. This was a cross-sectional study which was conducted at the University of Babylon – Hammurabi college of medicine and the college of dentistry during the academic year 2019-2020.

The research tool of the study questionnaire was adopted from variables of different studies.

Data were collected using the self-administered questionnaire which was adapted from another previous studies (18,19,20,21). The questionnaire consisted of socio demographic, previous experience of scientific research, knowledge and attitudes toward research, and perceived barriers.

Data was analyzed using SPSS version 21.

**Results**

Table 1 shows that 55.3% of the students have efficient internet connection 1.5% have no internet whatsoever. Figure (1) reveals female male ratio 2:1

Table 2 shows the distribution of 206 participates according to their skills in English language, less than one third of the students mentioned that they are good in writing and speaking English language.

Table 3 depicts the positive attitudes toward research.

Table 4 explains that only(34.5%) mentioned that they received encouragement from their tutors to participate in research activity while one-fifth of them agree that tutors are easily available to supervise research. Only 8.7% of the participants believe that there are chances are available to involve in research.

Regarding the obstacles table 5 shows that only (15.8%) cited that they have easy access to scientific journals and 72.3% of them said that there was no enough training activities in research and only 7.5% of the respondents had participated in some sort of research activities.

Table 6 shows that 85% of students believe that research is important for their future medical practice.

Table 7 reveals that only 11% don’t know the importance of studying the research methodology in the curriculum as a goal for their future career and self-learning.

More than halve of the study group (57%) believe research will be a career goal.

Figure (2) shows that 88% of the respondents either disagree or they don’t know that there are awards to encourage researcher.

Figure (3) shows that 3% of participant know that the government support research activities in Iraq.

<table>
<thead>
<tr>
<th>Internet Connection (total N=206)</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No internet</td>
<td>3 (1.5)</td>
</tr>
<tr>
<td>Slow connection (not always available)</td>
<td>89 (43.2)</td>
</tr>
<tr>
<td>High speed connection</td>
<td>114 (55.3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>English language skills – Writing (total N=206)</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very poor</td>
<td>7 (3.4)</td>
</tr>
<tr>
<td>Poor</td>
<td>4 (1.9)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>137 (66.5)</td>
</tr>
<tr>
<td>Good</td>
<td>47 (22.8)</td>
</tr>
<tr>
<td>Very good</td>
<td>11 (5.3)</td>
</tr>
</tbody>
</table>
### Table 2: Distribution of 206 participate according to their skills in English language (writing, reading, and speaking)

<table>
<thead>
<tr>
<th>English language skills – Speaking (total N=206)</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very poor</td>
<td>6 (2.9)</td>
</tr>
<tr>
<td>Poor</td>
<td>32 (15.5)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>122 (59.2)</td>
</tr>
<tr>
<td>Good</td>
<td>37 (18)</td>
</tr>
<tr>
<td>Very good</td>
<td>9 (4.4)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>English language skills – Reading (total N=206)</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very poor</td>
<td>5 (2.4)</td>
</tr>
<tr>
<td>Poor</td>
<td>11 (5.3)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>110 (53.4)</td>
</tr>
<tr>
<td>Good</td>
<td>63 (30.6)</td>
</tr>
<tr>
<td>Very good</td>
<td>17 (8.3)</td>
</tr>
</tbody>
</table>

### Table 3: Distribution of students according to their attitude towards conducting research during undergraduate study.

<table>
<thead>
<tr>
<th>Research involvement status (total N=206)</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not interested in research</td>
<td>55 (26.7)</td>
</tr>
<tr>
<td>Interested but don’t know how to involve in research</td>
<td>108 (52.4)</td>
</tr>
<tr>
<td>Actively looking to involve in research</td>
<td>43 (20.9)</td>
</tr>
</tbody>
</table>

### Table 4: Distribution of students according to exposure to encouragement to conduct research by their tutors

<table>
<thead>
<tr>
<th>Encouragement by academic staff to participate in academic research (total N=203)</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Encouraged</td>
<td>70 (34.5)</td>
</tr>
<tr>
<td>Not encouraged</td>
<td>133 (65.5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tutors are easily available to supervise research (Total N=204)</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Don’t know</td>
<td>71 (34.8)</td>
</tr>
<tr>
<td>Disagree</td>
<td>42 (20.6)</td>
</tr>
<tr>
<td>Average</td>
<td>50 (24.5)</td>
</tr>
<tr>
<td>Agree</td>
<td>41 (20.1)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Many chances are available to involve in research (Total N=206)</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Don’t know</td>
<td>70 (34)</td>
</tr>
<tr>
<td>Disagree</td>
<td>78 (37.9)</td>
</tr>
<tr>
<td>Average</td>
<td>40 (19.4)</td>
</tr>
<tr>
<td>Agree</td>
<td>18 (8.7)</td>
</tr>
</tbody>
</table>
Table 5: Distribution of students according to the access to scientific journals and capacity building in research methodologies during their undergraduate study.

<table>
<thead>
<tr>
<th>Easy access to scientific journals through university library (Total N=202)</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Don’t know</td>
<td>40 (19.8)</td>
</tr>
<tr>
<td>Disagree</td>
<td>84 (41.6)</td>
</tr>
<tr>
<td>Average</td>
<td>46 (22.8)</td>
</tr>
<tr>
<td>Agree</td>
<td>32 (15.8)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>There are enough training in research methods (Total N=198)</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strongly disagree</td>
<td>38 (19.2)</td>
</tr>
<tr>
<td>Disagree</td>
<td>97 (49)</td>
</tr>
<tr>
<td>Neutral</td>
<td>47 (23.7)</td>
</tr>
<tr>
<td>Agree</td>
<td>15 (7.6)</td>
</tr>
<tr>
<td>Strongly agree</td>
<td>1 (0.5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Participated in training course about medical research (Total N=200)</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strongly disagree</td>
<td>58 (29)</td>
</tr>
<tr>
<td>Disagree</td>
<td>107 (53.5)</td>
</tr>
<tr>
<td>Neutral</td>
<td>20 (10)</td>
</tr>
<tr>
<td>Agree</td>
<td>10 (5)</td>
</tr>
<tr>
<td>Strongly agree</td>
<td>5 (2.5)</td>
</tr>
</tbody>
</table>

Table 6: distribution of students according to their believe that research is important for medical practice and academic study.

<table>
<thead>
<tr>
<th>Research is important in medical practice (total N=201)</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strongly disagree</td>
<td>7 (3.5)</td>
</tr>
<tr>
<td>Disagree</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Neutral</td>
<td>21 (10.4)</td>
</tr>
<tr>
<td>Agree</td>
<td>69 (34.3)</td>
</tr>
<tr>
<td>Strongly agree</td>
<td>102 (50.7)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Research is important during academic study (Total N=202)</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strongly disagree</td>
<td>9 (4.5)</td>
</tr>
<tr>
<td>Disagree</td>
<td>7 (3.5)</td>
</tr>
<tr>
<td>Neutral</td>
<td>29 (14.4)</td>
</tr>
<tr>
<td>Agree</td>
<td>82 (40.6)</td>
</tr>
<tr>
<td>Strongly agree</td>
<td>75 (37.1)</td>
</tr>
</tbody>
</table>
Discussion

In developing countries, medical students are less involved in research activities owing to limited resources and other barriers.22

Attitude of undergraduate medical students and other students in health science, their knowledge about research and the barriers toward this educational activity are three key components that have an impact on research success.23 In this study there is a positive attitude toward research conducting among the study group, this finding goes with the finding of other studies (16,18,19). Positive attitude among the study group indicates very good sign to encourage them in this crucial field because poor attitude regarding conducting research act as barrier in planning and implementing research activates.23

English language skills are stronger barriers together with, lack of resources that facilitate the process of conducting research such as high speed internet, award and encouragement in the teaching environment to enhance research work as a learning tool that stimulating critical thinking and help them to gain critical appraisal skills that help in understanding the evidence medicine practice in their future career, these findings are similar to the findings reported by other researchers (16, 18, 19).

One of the main barriers toward research in this study current study is the inappropriate internet network connection which may affect the research activities.

In a local study the respondents think that the problems of slow network speed and frequent interruptions in communication are obstacles that cause problems in conducting academic research.24

In this study only one fourth of the respondents mentioned that they receive support from their mentors to bring their attention to the importance of the skills of conducting research in practicing medicine, mentorship is crucial for research.25 From our experience under graduate students who were encouraged by their professors were publishing scientific papers.26,27,28,29,30

The lack of adequate mentorship has been reported as a main research barrier in many studies.15,21

Most participants think that research mentors are unavailable, and a lack of guidance and supervision was reported by a substantial number of participants as a reason for not participating in research, lack of academic staff and institutional support in promoting health research is an important obstacle, in a local study about the status of scientific research and the obstacles according to the opinions of 600 academicians from six Iraqi universities mentioned that the absence of institutional support for the scientific research and the low level of governmental expenditure on scientific activities are the main barriers for conducting scientific research.31

Conclusion

Students had the moderate level of knowledge and positive attitudes toward the conduct of medical research. English language funding, awarding, poor internet connection and limited access to relevant medical journals and databases were the major barriers.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Babylon – Hammurabi Medical College, Iraq and all experiments were carried out in accordance with approved guidelines.

References

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Study of Some Hematological and Biochemical Parameters in Children with Thalassemia in City of Karbala

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1Msc in Biology. Alsafwa University College, 2Msc in Biology. Alsafwa University College,
3Msc in Biology

Abstract

This research including the study of some hematological and biochemical changes in children suffering thalassemia in the province of Karbala.

To achieve this aim 50 samples was arranged in two groups first group include 40 patient with thalassemia male and female attending Imam Hussein medical city and second group include 10 healthy children as control.

The results appear decrease in concentration of packed cell volume (PCV), Red Blood cell count and Hb. Also the results appear decrease in biochemical parameters represented by (GOT) and (GPT). While there is increase in WBC count in patients as compared with control. The result also shown that thalassemia mostly occurs in male than in female.

Keywords: Thalassemia, Packed Cell Volum(PCV), Glutamate oxaloacetate Transaminase(GOT), Glutamate Pyruvate Transaminase(GPT)

Introduction

Thalassemia is a group of inherited autosomal recessive blood disorders that originated in the Mediterranean region. In thalassemia the genetic defect, which could be either mutation or deletion, results in reduced rate of synthesis or no synthesis of one of the globin chains that make up hemoglobin.¹

Abnormalities in hemoglobin, resulting from the deficiency or complete absence of one or more of the globin chains, give rise to the thalassemia. Patients with thalassemia suffer from varying degrees of anemia, that is, a shortage of red blood cells. (2, 3)

Classification of thalassemia: The two main types are called Alpha and Beta thalassemia, depending on which part of globin chain is produced in reduced amounts. Normally, alpha globin chain is made by four genes (two from each parent), two on each strand of chromosome 16. The alpha thalassemia are caused by a decrease in production of alpha globins chains due to deletion or mutation of one or more of the four alpha globins genes located on chromosome 16.⁴

Unlike the deletion that constitute most of the alpha thalassemia syndromes ,beta thalassemia are caused by mutation on chromosome 11 that affect all aspect of beta globin production : transcription ,translation , and the stability of the beta globin production. There are more than 200 of mutation within the beta globin gene found worldwide to produce beta thalassemia ¹ .Beta thalassemia includes the following two forms:

A- Major Thalassemia (Cooley’s anemia): The child born with major thalassemia has two genes for beta thalassemia and abnormal beta-chain. Inherit the defect gene from both parents. A person who is infected with this type showing clear symptoms of the disease since childhood ⁵.

B- Minor Thalassemia The individual with minor thalassemia has only one copy of the beta thalassemia gene (together with one perfectly normal beta-chain gene) occurs when received the faulty gene from only one parent. The Person have symptoms of simple anemia and be able to transmit the disease to his sons ⁶.
Material and methods:

The study included 40 patients suffering from thalassemia and 10 healthy children as control attending Al-Hussein medical city. Venous blood samples (5ml) are obtained from all patients and controls. 2ml was collected in EDTA tube and the remaining 3ml collected in plain tubes for serum collection.

Determination of hematological parameters:

Hemoglobin (Hb), red blood cells, white blood cells and Packed Cells Volume (PCV) were measured by complete blood count (CBC) apparatus.

Measurement of the enzyme GOT & GPT:

GOT & GPT enzymes were measured by using the colorimetric method depending on the kit of kind (Syrbio).

Results

Table (1) show the range and mean ± SD for some hematological changes for patients with thalassemia as comparison with healthy children.

<table>
<thead>
<tr>
<th>Pcv %</th>
<th>Hb g/dl</th>
<th>RBCs 106/ul</th>
<th>WBCs 103/ul</th>
</tr>
</thead>
<tbody>
<tr>
<td>children</td>
<td>children</td>
<td>children</td>
<td>children</td>
</tr>
<tr>
<td>patients</td>
<td>Healthy</td>
<td>patients</td>
<td>Healthy</td>
</tr>
<tr>
<td>(24.79-31.86)</td>
<td>(43.69-44.46)</td>
<td>(7.24-9.63)</td>
<td>(12.11-13.56)</td>
</tr>
<tr>
<td>27.48 ± 2.17</td>
<td>44.03 ± 0.28</td>
<td>8.30 ± 0.78</td>
<td>12.59 ± 0.57</td>
</tr>
<tr>
<td>7.24 ± 3.80</td>
<td>5.78 ± 2.18</td>
<td>4.63 ± 0.08</td>
<td>7.24 ± 3.80</td>
</tr>
</tbody>
</table>

Table (2) show the range and the mean ± SD for some biochemical changes for patients with Thalassemia as comparison with healthy children and

<table>
<thead>
<tr>
<th>GOT Unit/ml</th>
<th>GPT Unit/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>children</td>
<td>children</td>
</tr>
<tr>
<td>patients</td>
<td>Healthy</td>
</tr>
<tr>
<td>(12.18-18.31)</td>
<td>(23.4-53.4)</td>
</tr>
<tr>
<td>14.38 ± 1.54</td>
<td>32.11 ± 10.27</td>
</tr>
<tr>
<td>(14.14-22.7)</td>
<td>16.92 ± 2.10</td>
</tr>
<tr>
<td>(50-57.6)</td>
<td>54.52 ± 2.19</td>
</tr>
</tbody>
</table>
Discussion

The results obtained from our study indicates as shown in Table (1) to decrease the rate of both the packed cell volume and the concentration of hemoglobin and the number of red blood cells in the infected children compared to healthy children. The reason that the rate of the packed volume of the cells depends mainly on the number, size and shape of RBC and on the range blood viscosity and his easing.7 As well as the reason for the lack of red blood cells, where the age of pellet of red blood normal 120 days. When dissolved, and eat up by the reticule-endothelial system 8 but when you get a pathological case such as thalassemia, the bone marrow is unable to compensate for lack RBCS so hold a total of red blood cells as quickly break it, so the numbers of red blood cells remain diminish so lead to this characteristic pallor and yellowing (jaundice) of the skin clearly 9.

The decline in the level of hemoglobin in patients as compared to control due to the rapid decay of red blood cells, and therefore decrease the amount of processed hemoglobin body 10.

The results obtained also indicate as shown in Table (1) to increases in rate of total white blood cell in the affected kids, Maybe the reason is due to the high crash blood cells inside and outside the bone marrow, causing a severe decrease of partial pressure of oxygen and this is a motivating factor for the production of the hormone Erythropoietin kidney. This hormone stimulates the bone marrow to increase hematopoietic formation through the conversion of undifferentiated cells into differentiated cells in a tissue bone marrow including white blood cells 11. Moreover, the start of some liver cells and some of the spleen cells configured centers to generate cells blood 12.

Through the results as shown in Table (2) note down some of the biochemical changes in the serum of patients with thalassemia both enzymatic GOT, GPT, compared with healthy children if both spreads enzymes in many tissues of the human body 13, that the reasons for the low effectiveness of the enzymes from the normal level of healthy people this may be due to the amount of iron found in the serum of patients, which are deposited in these organs, which results in breaking down some of the fat cells of these organs 14.

The result show the relationship between the incidence of disease, thalassemia and sex Figure (1) is observed that the number of infected males more than females and as the genes responsible for the production of the common Peptides strings in build hemoglobin located on chromosomes 11 and 16, 15 and not found in chromosomes x, y not found correlation between sex and the infection.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.
Ethical Clearance: All experimental protocols were approved under the Alsafwa university college and all experiments were carried out in accordance with approved guidelines.

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A Comparative Study in Psychological Burning among Football Referees And Football Referees of First Class Halls in Southern Zone in Iraq

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2 Assistant Prof., University of Thi-Qar, College of Physical Education and Sports Sciences

Abstract

The research aimed to know about differences in the phenomenon of psychological burning among football referees and football referees of first class halls in southern zone. The researchers assumed that there are differences with moral guidance in the phenomenon of psychological burning among football referees and football referees of first class halls in southern zone. The researchers had mentioned to the theoretical studies that contained of multiple researches related to the topic of the research. The researchers used the describing method by principle of comparative studies due to is suitable for the problem of the research. The research sample was contained from (28) referees divided onto (14) football referees and (14) referees of first class halls after implementing measure of psychological burning on a sample of research the researchers did to find differences between the two groups by using the law of (T) for the independent samples, After that these results has been previewed and analysis and discussed so the researchers reached to conclusions the most important of the is that there are no any moral differences between the groups of football referees and football referees of first class halls in southern zone.

Keywords: Burnout, football referees, football

Introduction

Preface and importance of the research.

Psychology is to be considered of important sciences that needs study and big deepening due to is has big biological effecting on individuals 1, So it is a science which is so needful in last years especially in the sport field due to it has active effecting whether in training or competing some gave it priority in classification 2 because it effects on the physiological sides of different body organs that do an active role in performance so it has been repeated in last years the term of (Burnout) within the terms of sport psychology which means (burning) where it is the status of physical and psychological exhausting that may infects the athletic due to accumulating of loads of ambition and entertainment and a source for worry and fear instead of desire in superiority, win and highness.

Refereeing process is to be considered a difficult mission the referee bears heavy loads and many troubles but he feels delighted to practice it because the basis of practicing is a pure hobby so he seeks always to success through continuous work despite it has physical, technical and social obligations and this the top 3 of the evolution and success to reach top levels and big degrees.

So the occupation of referee is most occupations that being subjected to psychological stresses and these stresses by its turn causes psychological burning and the psychological burning leads to creating negative situations against others which would leads to a case of restless and self conflict and down fall of personal relations where it effects on the personality in all ad this effect would be more clear in the psychological and

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physical health and performance in work which will causes that the referee will not be able to do his role in complete that participate in his feeling to be disable and gloominess with exhausting effort leads to a case of exhausting and psychological exhausting the thing that will enforce him to performs with low than its required from him ⁴.

The football game and football for halls are most popular games in the world and the role of the referee would be considered very important in managing the wheel of the match and to lead it to the safe lands and this requires high physical and psychological effort where the nature of psychological stresses that are being faced by the referee whether they where from the audience or press or media means , competing teams or referee committee that he works with them gives and big importance to be studied , Due to its role in rising the level of referee occupation and developing it and to rise it to the required level which will make practice of this occupation clear of problems and to get rid of its negatives that which will maintain giving his value and respected status for the sport referee in the society ⁵.

Through what has been mentioned and in view of available scientific resources about this important psychological phenomenon so the importance of the current research shows clearly in dealing with one of the sport jobs that impacts on sport field and joins with others in showing level of matches by shining & honoring vision for any sports activity being practiced , from here the importance of the research come out to know the phenomenon of psychological burning of football referees and football referees of fist class halls in order to know the referees of any of these two games are more subjecting to the psychological burning other than others. For sake to reduce the severity of psychological burning in them through knowing the reasons that causes that caused their burning.

Problem of the research:

Few of people knows the size of big work that has been assigned on the shoulders of the referee whether before or after or during the match so he works under big stresses despite multi global and Arabic studies that took the phenomenon of psychological burning in the sport field but most of them ( according to the acknowledgment limits of the two researchers ) has not mentioned to the psychological burning of the sport referee especially foot bal referees and football referees of halls which may play an important role in leading the match and give it big beauty , through observation and private meeting for some of the referees and following by the researchers of the periodic and referees the problem of the research appeared that there are a number of referees does not continues in work due to being subjected for some psychological stresses or they may continue in their work with no acceptance about this work and then falling of their level of achievements , These stresses that are causing psychological burning varies by the difference of the game and its requirements which has pushed us to try to explore about these differences in the phenomenon of psychological burning of them.

Goal of the research:

Identification on differences in the phenomenon of psychological burning among football referees and football referees of first class halls in southern zone.

Supposition of the research:

There are differences with moral guidance in the phenomenon of psychological burning among football referees and football referees of first class halls in southern zone.

Areas of research :

1 Human field: - football referees and football referees of first class halls in southern zone – republic of Iraq

Time field 15/5/2018-1/2/2019

Location field, Stadiums and halls of football in southern zone

Method of research and its field procedures:

Research method the two researchers used the describing method by principle of comparing due to its suitable for the research problem.

Society of the research and its sample

The society of the research has been formed from football referees and football referees of first class halls in southern zone ( Basra – THI-QAR – Maisaan , Mothanna) and those who had been really recorded in the union of the football federation who are (39) referees of football and ( 28) referees for halls football , but the sample of the research has been chosen by random method where (28) referees had been chosen with reality
of (14) referees for football and (14) referees for first class halls and the table (A ) represents some of the information about society and sample of the research.

**Table 1. Indicates number of society and research sample**

<table>
<thead>
<tr>
<th>Number Province</th>
<th>Number of football referees</th>
<th>Number of referees of halls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total number</td>
<td>Sample</td>
</tr>
<tr>
<td>Basra</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>THI-QAR</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>Maisaan</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Muthanna</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>14</td>
</tr>
</tbody>
</table>

**Research tool :**

**Psychological burning measurement of the sport referee :**

The measurement has been designed by ROBERT Whiteburg and Biggi Richardson to measure the feeling of the sport referee in burning under title of burning in refereeing and it is a measurement being abstracted from list of ( Masilas) of burning and has been translated to Arabic Mohammed Hasan Alawi, The measurement includes (15) phrases the referee answers on the item of the measurement according to seven substitutes (never implemented on me, implements on me very little, implements on me little, implements of me middle, implements on me with large size, implements on me with very large size, totally implements on me) the degrees from (1 – 7) is being given respectively except items (11.7.5) where their degrees is to be counted reversely. As much the total referee degree comes close from (105) it means he reached the degree of burning appendix (1)

**scientific specifications of the measurement**

**Credibility of the measurement:**

The two researchers had depended on measurement outward credibility and that a group of specialists to evaluate the validity of the measurement to measure the mark that it has been made for it, so the researchers did showed the measurement on a group of specialists appendix (2) in the field of sport psychology and football to go over the items of the measurement and to indicate how far is the validity and after collecting the forms from the specialists those forms has been emptied by the two researchers where the ratio of the agreement (100%)

**Measurement stability:**

The tool would be considered stable if it gives same results if measures the phenomenon for several times where the method of measurement stability through retesting is most used methods in finding stability factors and most common and for sake to get of measurement stability the researchers has divided the measurement on a sample consisted of (8) referees from those who do periodic first class football teams and football for the halls and not from oral test sample then the same test repeated after two weeks from the first test on same sample after collecting the data they had been treated statistically by using the factors of simple connecting and the value of (R) 0.91 and this proofs that there is high connection and then excitant of stable factor of the measurement.

**Statistical means:-**

The following statistical treatments had been used:

1. Accountant media
2. standard deviation
3. difference factors
4. simple connection factors
5. T Test for independent samples

3-preview results analysis & discussing them:

3-1 preview and analysis the results of psychological burning measurement

3-1-1 among football referees and football referees of halls

Table(2) Shows the differences in psychological burning among football referees and football referees of first class halls in southern zone

<table>
<thead>
<tr>
<th>Treatments Changes</th>
<th>Football Referees</th>
<th>Football Referees of halls</th>
<th>Value of T</th>
<th>Statistical guidance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S +/- A</td>
<td>S +/- A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psychological burning</td>
<td>45.21 11.15</td>
<td>49.66 7.41</td>
<td>1.199</td>
<td>2.06 Non moral</td>
</tr>
</tbody>
</table>

Value of (T) table at degree freedom (26) and the level of guidance (0.05) is (2.06)

Table (2) indicates the accountant middle and standard deviations and value of (T) accounted in the measurement of psychological burning of football referees and football referees of halls in the southern zone, results showed that then accountant middle of the group of football referees where (45.21) with standard deviation (11.15) but the accountant middle of the group of referees of halls where (49.66) with standard deviation (7.41) but the value of (T) accounted where (1.199) which is less than (T) table value of (2.06) with freedom degree (26) and below level guidance of (0.05) and this proofs that there are no any moral differences among football referees and football referees of halls in the psychological burning.

Discuss the Results

It is clear from the table (4) that there are no any moral differences in the results of the measurement of psychological burning among football referees and football referees of halls in the southern zone the researchers justifies that it belongs to the good preparation and programmed training by the referees whether football referees or football referees of halls to reach by their selves to high level of physical fullness that enables them to good performance along the time of match as well as big desire by referees for this job and their love to it also the personal experience of those referees and their understanding of the items of the international law in managing the match and how to deal with the players in good manner as well as the exchanged cooperation between the referee and his associates in leading the match has caused not excitant of phenomenon of psychological burning for them … and this is what has been mentioned by AMAL ALI SALOOMI (2000) that the control on the burning requires from the athletics that they are aware of resisting against the burning where it is possible that they will analysis with constructive form and conveys their feelings to the others and by that they are reducing the burning. defined steps that leads to reduce the burning, The athletic or the referee can learn principles of psychological stress and get benefits by close examination, relaxing and others also knowing stresses is the side that enable the athletic to reduce traces of burning also that the best technique for the athletic is to identify on the problem and bearing it as much as he can then reducing it and then removing it.

Conclusions

No existent of moral differences between the two research groups of referees of football and football...
referees of halls of first class in southern zone.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Thi-Qar, College of Physical Education and Sports Sciences, Iraq and all experiments were carried out in accordance with approved guidelines.

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Drug Addiction and the Most Important Diseases that affect Human Mental, Mental and Physical Health in Iraqi Society

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Abstract

First: Problem and Importance of search

The effects of psychological, health, mental and physical are serious diseases that affect the individual drug user was not the drug of this age has been known by man since ancient times and fought ancient societies medically, culturally, socially and religiously Human life from the deterioration of his health and his illnesses complex mental health, mental and physical

Second: Goals of search

Drug addiction and the most important diseases that affect human mental, mental and physical health in Iraqi society.

Third: Society and sample of search

The study sample was selected on two middle schools in Al-Hilla city (Imam Ali School for Boys and Bint Al-Huda School for Girls). 100) Researched and used the method of social survey method intentional sample and questionnaire, interview, observation, participation and statistical methods and hypotheses and the most important treatments for addicts are psychological, health, mental, physical and social treatment.

Keywords: Drug addiction, human mental, physical health

Introduction

The problem of drugs is one of the most important problems that cause great danger to the future of society, because they have individual and social effects, and the society is responsible for it as a part of its wealth. Since ancient times and thousands of years, mankind has known some plants and herbs to use them as medicine. Drugs were not born of this era it has been known to human since the ancient times and fought by ancient societies culturally, socially and religiously, Where drugs are the most serious disaster known to humanity in its history and began to evolve into an illegal international trade sponsored by organized gangs aimed at destroying the energies and capabilities and values of the youth of society which it is true treasure and this means emptying the community of creative minds in it so that our backward society remains easy to control. For decades, Iraqi society has faced fierce attacks that are trying to overthrow Iraqi society and destroy all its capabilities. These attacks are an attempt to destroy Iraqi society, especially the youth and adolescents who are more vulnerable than others to receive everything new. In spite of all attempts, efforts must be made to combat the preservation of the entity and build a good society and having a valuable system. Drug addiction and the most important diseases that affect human mental, mental and physical health in Iraqi society. The problem is known linguistically: “The problem and the problem are all problems and problems of difficult and code ¹. The problem defines a idiom: it is a state of form and confusion, some puzzling or searching to get rid of this situation, replacing it with a feeling case of satisfaction and agreement². Drugs are linguistically called: They are all substances that lead to relaxation, debility, transport and sleepiness. They also reduce or eliminate pain. It is an incomplete definition. If we take this definition with, opium, morphine and heroin are drugs but the class of stimulants such as amphetamine or hallucinogens (LSS) cannot be placed under this definition ³. Drugs legally:
They are the materials specialized by the law of each country in special schedules, which the authors of these laws believed that it is harmful to the individual, group or nation. The United Nations Commission on Narcotic Drugs knew that it was any raw or prepared substance containing hypnotic and analgesic elements that, when used in non-medical industrial conditions, would lead to a state of habitual or addictive addiction to the individual and society, physically, psychologically and socially.

Factors leading to drug abuse.

First: The Economic Factors

1-The high standard of living, which receives a lot of burdens on the individual makes him incapable of it, which leads him to abuse trying to escape from his social reality.

2- Unemployment as a result of the great pressures placed on difficulty of life and as a result

3-Increasing the requirements of life in general where there are no longer called so-called luxury needs, but have become necessary.

Second: Psychological factors: There are a group of psychological factors exert a great impact on the person forced him making him giving up to the difficulty, the result of that is the practice of escaping from the reality to drug abuse or both:

1-The many psychological pressures resulting from frustration because it is not achieved for a particular desire or need.

2-Feeling of a deficiency compound due to a certain disability, handicap or failure to match others at certain levels of class, cultural or other.

3-Feeling of failure, inability and no efficiency.

Thirdly: Social factors:

1-Meeting or accompanying bad companions: The collection of psychological and social studies conducted on the causes of drug abuse with these friends.

2- The feeling of emptiness: There is no doubt that the existence of the vacuum with the availability of places that are good for the energy of young people.

3- Traveling abroad: There is no doubt that traveling abroad with all the means of flattering and for drug abuse.

4-Excessive cruelty to children: It is one of the things almost gathered by the scholars of reprimand, then reflected on his, sin and drug abuse which can find it.

5-The influence of the media: The media have a large role in the members of society, especially the youth group.

6-The absence of the school’s message: This is the responsibility of the director or teachers and should be withdrawn from corruption and vices.

The second topic: the most important effects and problems of drug abuse

The social effects of drug addiction are:

1-The abuser becomes a bad example to be followed by his family and society.

2- The possibility of transferring the abuser to the family members through imitation and simulation.

Second: The economic effects of the addiction:

1-Waste funds and large human resources in order to deal with this, or spend money by the state and the concerned parties.

2-Damage to the national product of the father because of the trade activities prohibited by law.

A-The economic effects on the level of the individual: that the addiction to a large impact on the individual ones lead to the destruction of money and the children’s food and perhaps a deviation to the path of dishonesty, social and economic damage.

B- Economic Impacts at the State Level: Most of the world’s countries produce drug consumption, which is an organized public trade bout $ 120 billion annually on accidents, crimes and diseases. One of the most dangerous effects of addiction on the role of economic activity as follows:

- Third: the impact of drugs on society:

1-Addiction affects the modesty of the community may show the crimes of rape, assault, murder and car accidents.

2-The spread of infectious diseases and the most serious AIDS.
Fourth: Health and psychological effects ²⁰:

1- Psychological and mental disorders due to drugs effect on the nervous system.

2- Drug abuse leads to a general disorder of thinking and difficulty ²¹

Chapter Four: Field Research Procedures

First: Research Methodology.

1- Social Survey Methodology:

A- Comprehensive survey: which conducts a comprehensive study of all the subjects of society through.

B- Survey method of the sample: In which the researcher only study a specific number of cases or subjects within the time and effort and possibilities available to the researchers ²².

Current research objectives which require a community and a search sample.

1- The research community ²³.

1- Social researchers often find it difficult to conduct a comprehensive field study of all the items of the research part of the community keen to accurately represent the characteristics and components of research ²³.

2. Sample of the research: A sample of (100) respondents was selected from the research community. For the purpose of achieving the current research, the questionnaire tool was to be distributed to the respondents in two preparatory schools and for the final stages only in Hilla city: Imam Ali School for Boys, Bin al-Huda School for Girls ²⁴.

Fourth: Fields of research: In each field study areas of study should be identified and clarified by the researcher, these areas are centered in three aspects is the human, geographic and temporal, it can be clarified these areas as follows:

• Human field: means the individuals to whom the field study will be conducted, the human field is in the city of Hilla for preparatory schools (Imam Ali school for Boys - Bint Al Huda school for girls) is a model.

• Time field: means the length of time spent in the collection of information and data related to the study, and the field study process for the study sample took a period of time from 11/1/2019 to 27/3/2019. only.

• Statistical methods in the process of describing and analyzing research data ²⁵:
  - 1- percentage(%)  
  - =\frac{\text{part}}{\text{All}} \times 100
  
  Table (1) shows the number of family members of the respondents

<table>
<thead>
<tr>
<th>Num. of family</th>
<th>Num.</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-2</td>
<td>20</td>
<td>20%</td>
</tr>
<tr>
<td>4-5</td>
<td>38</td>
<td>38%</td>
</tr>
<tr>
<td>6-7</td>
<td>26</td>
<td>26%</td>
</tr>
<tr>
<td>8-9</td>
<td>16</td>
<td>16%</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100%</td>
</tr>
</tbody>
</table>

It is clear from the table above, the second category (4-5) the percentage was (38%), percentage (16%). We conclude from this that the age group of (4-5) took more percentage .

Table (2) shows the monthly income for the Family

<table>
<thead>
<tr>
<th>Monthly income for the family</th>
<th>Num.</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than needed</td>
<td>8</td>
<td>8%</td>
</tr>
<tr>
<td>Fill the need</td>
<td>72</td>
<td>72%</td>
</tr>
<tr>
<td>Overflowing</td>
<td>20</td>
<td>20%</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100%</td>
</tr>
</tbody>
</table>
The above table shows that the monthly income of the research sample, that covers the need, with percentage reached (72%), the latter rank is lesser than the need with (8%).

**Table (3) shows that drug abuse leads to family problems of the respondents**

<table>
<thead>
<tr>
<th>Addiction abuse causes problems to the family</th>
<th>Num.</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>90</td>
<td>90%</td>
</tr>
<tr>
<td>No</td>
<td>2</td>
<td>2%</td>
</tr>
<tr>
<td>Not always</td>
<td>8</td>
<td>8%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100</td>
<td>100%</td>
</tr>
</tbody>
</table>

The table shows that the drug abuse leads to the family problems, the respondents who answered (yes) were (90%). While those who answered (no) of the respondents were (2%).

**Table (4) shows the weakness of the religious faith of the respondents**

<table>
<thead>
<tr>
<th>religious faith</th>
<th>Num.</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>66</td>
<td>66%</td>
</tr>
<tr>
<td>No</td>
<td>14</td>
<td>14%</td>
</tr>
<tr>
<td>Perhaps</td>
<td>20</td>
<td>20%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100</td>
<td>100%</td>
</tr>
</tbody>
</table>

The above table shows that the weakness of the religious faith leads to the individual to come to drug abuse, those who answered (yes) of the respondents, were (66%), those who answered (no), the percentage was (14%).

**Table (5) shows that family disintegration is a driving force for drug abuse**

<table>
<thead>
<tr>
<th>family disintegration force for drug abuse</th>
<th>Num.</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>52</td>
<td>52%</td>
</tr>
<tr>
<td>No</td>
<td>14</td>
<td>14%</td>
</tr>
<tr>
<td>Not always</td>
<td>34</td>
<td>34%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100</td>
<td>100%</td>
</tr>
</tbody>
</table>

The table above shows that family disintegration. The number of respondents with “yes” are (52%). While those who responded with “no” by (14%).
Table (6) shows the most important factors leading to drug abuse.

<table>
<thead>
<tr>
<th>Factors driving for drug abuse</th>
<th>Num.</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Economic</td>
<td>26</td>
<td>56%</td>
</tr>
<tr>
<td>Social</td>
<td>22</td>
<td>22%</td>
</tr>
<tr>
<td>Psychological</td>
<td>48</td>
<td>48%</td>
</tr>
<tr>
<td>Religious</td>
<td>4</td>
<td>4%</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100%</td>
</tr>
</tbody>
</table>

The table above shows that the factors leading to drug abuse were (48%). Those who responded to religious factors were (4%).

The results of the research and the most important treatments and recommendations to address the problem of drugs and the most important steps to prevent children from abuse

First: Search Results: The research resulted in a number of results, the most prominent of which were:

1- It was found that 20% of the respondents answered that the level of income is over their needs.

2. It was found that (90%) of the respondents have answered that drug abuse leads to family problems.

3- The results of the field research revealed that (66%) said that the weakness of the religious.

4- It was found that the most important factors that drive society members to drug abuse are psychological factors, which came first and by (48%).

5- It was found that the use of drugs leads individuals to use the crime and a percentage of (68%) as a result of loss of feeling and lack.

Second: The most important preventive measures

1- Enhancing the capabilities of the specialized security forces in the fight against drugs, especially at border ports, ports and airports.

2- Participation of members of the security forces and police services in training courses.

3. Encouraging the citizen to report and provide information about those involved in drug crimes.

4- Enhancing the effectiveness of coordination between the security agencies to monitor.

5- Coordinating anti-narcotics efforts through continuous exchange and organizing efforts in this field.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Babylon University, Faculty of Arts, Iraq and all experiments were carried out in accordance with approved guidelines.

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Human biotoxic, Human Reproduction Effects and Biomarkers to Assess the Health of the Ecosystem by Estimate the Ratios of Heavy Elements

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College of science. University of Alqadisiyah

Abstract

This study was conducted to investigate the concentration of some heavy metals (Cr, Zn, Cu, Cd, Pb) in some types of frozen and canned fish in Diwaniyah. Five types of fish were widely available (Stromateus Sp., Parastromateus niger, Labeo rohita, Sardina pilchardus). And the number of replicates of one species, where the number of fish during the study period 20 fish. The results obtained showed a difference in results and the concentrations of the heavy elements above were measured by ppm (0.231-13.18 / 0.012-0.915 / 0.028-0.0234 / 0.400-2.161 / 0.021-0.141) for Cr, Zn, Cu, Cd and Pb respectively. The above results showed the increase in concentrations of heavy metals compared to previous studies, as well as their comparison with global and local determinants of these elements. The concentrations of heavy elements in the tissues of the muscles and muscles of the Stromateus Sp fish (0.30, 0.89, 0.89, 0.89, -0.59 / ND / 0.100) for Cr, Zn, Cu, Cd and Pb respectively. But in gils and frozen erythematic muscles were (0.2 / 0.473 - 0.019 / 0.043 - 0.053 / 0.115 - 1.589 / 0.929 - ND / 0.100) for Cr, Zn, Cu, Cd and Pb respectively.

Keywords: Human biotoxic, biomarkers, health, ecosystem, heavy elements

Introduction

Studying and estimating trace element content in the living and non-living components of aquatic ecosystems helps to assess the level of pollution in them and reduce their spread and knowledge of their sources.

This type of contaminant enters the environment through two natural source and human source sources and is classified as Persistent Toxic Substances for the inability of microorganisms to decompose, as well as their accumulation and toxicity to living organisms. Their penetration through the components of the ecosystem is a serious and unavoidable future as well as threatening the living environment of aquatic organisms, possessing the ability to integrate and move in food chains, and then their concentration in living organisms with the presence of mortality, in some cases when they increase their concentrations beyond permissible limits. What is dangerous is the persistence of biotic activities and activities that transport these elements throughout the food chain, as well as the ability to bioaccumulate the food chain and affect the composition and diversity of the aquatic environment communities. And also have the capacity to form complex compounds with most organic and inorganic compounds found in organisms, leading to their accumulation in food levels increasingly from one level of food to another of these living ecosystems.

The transmission of this type of contaminant across the food chain and its accumulation in the body of organisms increasingly from one level of food to another in the aquatic environment is one of the most serious problems in the pollution of the aquatic environment. The first path is the concentration or aggregation of the elements by the living at all levels of the food chain. This process is carried out through a variety of methods, including entering through breathing to the fish or through the membrane of the body of the organism or by sticking to the body as in the zooplankton. The transition gets from As well as some species of fish, crustaceans, worms and grafts. The other way to transport these contaminants is by feeding each other through the different food chain, an important method that can reach the elements (Monperrus, et al., 2005). Therefore, the estimation of trace elements in the aquatic ecosystem is...
of great importance in determining and controlling their levels. Damage caused by contamination of trace elements in aquatic organisms is not limited to toxic and deadly effects, but also causes other damage, such as Mutagenic mutations, Embryo toxic and Gonadotoxic infections, and also reduced growth rates and disruption of processes Metabolic processes. They are also found in the bodies of living organisms, too, with very low concentrations, some of which are essential for sustaining their life, such as copper, zinc, iron and manganese. In general, these elements are important for building part of the body. They also play a role in regulating body fluids through ion exchange (Al-Tai, 1987), but high or low levels of the optimal limits lead to physiological damage and loss of life. Living organisms have the ability to absorb. The heavy elements dissolved in the water medium by some body tissues such as skin and skin. And its concentration in the liver, kidneys, muscles and muscles, leading to the accumulation of high concentrations in the body. These elements are dangerous even if they are found in low concentrations in the environment and when increased, lead to poisoning and death. (Park and Presley, 1997).

stressed that the level of toxic effect of these elements depends on the type of element and its concentration on the aquatic environment as well as the time of exposure to this element.

Sources of heavy metals:

There is a difference in the sources of heavy elements between the natural effects of volcanic eruptions, fires and sources of human activity such as household wastes and various agricultural activities such as pesticides and chemical fertilizers, as well as mining, mineral extraction, petroleum refining, and automotive and mining industries (Kuwait Institute for Scientific Research, 1989; Williamson et al., 1995) explained that the waste of battery factories is one of the most important sources of cadmium and lead in the aquatic environment. The atmosphere is an important source of trace elements due to the high tide of dust. Some trace elements in the air may increase their concentration more than 1000 times from their natural level as a result of human activity (9,10,11) notes that the Kafue River in Zambia is one of the rivers that suffer from degradation and the potential loss of biodiversity due to the change in the quality of water resulting from the use of pesticides containing heavy metals to eliminate algae. noted that the concentration of heavy elements in the environment can increase as a result of decomposition of aquatic organisms after their death. As well as the ability of high sediments to retain elements and contaminants and synthesize them in various mineral compositions in a way that is not ready (Hlavay et al., 2004). However, chemical and physical changes such as reaction, salinity, oxygen concentration and oxidative stress change, To return to the water medium, ie, the intervention of the geochemical cycle again, so that the sediment is an important source of water pollution (Cappuyns and Swennen, 2005; Audry et al., 2004).

The aims of study:

1. Determination of concentration of heavy metals (Cr, Zn, Cd, Pb, Cu) in different species of fish.

2. Comparison of the concentrations obtained from this research results of studies conducted in some countries.

3. Comparison of concentrations of these elements also allowed concentrations in international standards and standards.

Materials and Methods

Fish species studied:

1. Stromateus Sp. fish (silver)

Scientific Classification:

Family: Stromateidae

Genus: Pampus

Pampus argenteus

The species of fish follows the white Stromateus Sp species

Features and form:

These fish are characterized by the presence of a vesicle or chisel in the esophagus to grind the food and fall after the pharynx directly.

The body is flat and wide, fineless pelvic (pelvic), the scales are small circular, the body is silvery white and the head color is dark compared to the body (Coad, 2010)

2. The thickness of raho (or roho)

Scientific Classification:

Family: Cyprinidae
Genus: Labeo

*Labeo rohita* (Roho labeo)

These fish belong to the carpaceae. They have a large body length of 100-60 cm.

The head is moderate, the eyes are large round and have a single dorsal fin and a lower waist fin. Which are not threatened with extinction or extinction is very weak. (Dham, 1977)

The average length of the fish ranges between 30-23 cm and weight 113 g, body color from the top gray to blue and silver bottom color.

The fins are soft, non-forked, the caudal fin is deeply cleft, the scales are circular, the eyes are large, the mouth is frontal and the teeth are undistinguished or absent. (Coad, 2010)

5-mackerel Fish (bath thi-ckness):

Scientific classification

Scombridae Family:

Genus: Rastrelliger

Rastrelliger Kanagutra

Its body is rectangular and pressed, ranging in length between 20 - 65 cm, and may reach 70 cm. It has crusts even on the chest area except for a small area in front of the pelvic fin. Their color is silvery with a tinted tint between blue and green on the back on the body a lot of orange color spots. They feed on crustaceans and small fish, common in mid-depth waters, away from the shores of edible fish. (Dham, 1977)

* Cleaning Tools Lab wares cleaning:

Glass tools and polyethylene bottles were cleaned according to the method described (Boehnke & Delumyea, 2000). Wash the dishes thoroughly with tap water and cleaning powder and rinse thoroughly with water several times. The tools are then placed in a basin containing diluted hydrochloric acid at 10% concentration for at least 24 hours and then washed several times with distilled water (Nollet, 2007).

Collection of samples

In this study, five types of fish were widely available in the commercial markets and shops for the sale of frozen and canned fish and four replicates were taken for each type of different companies.

Extract trace elements from fish

After collecting the fish samples and transferring them to the laboratory, they were washed with distilled water. The fish were separated for the purpose of separating some of the organs (muscles and galsam). The muscle was taken from the left side after the head area of the fish. The tuna and sardines were mixed. For digestion of fish samples for the purpose of measuring the ion of trace elements in it and summarized as follows:

A dry weight of 0.5 g dry weight was taken from the fish tissue (muscle and galsum) after drying using the 80 ° C convection oven for 24 hours. After that, it was grinded and sifted with a 0.5 mm slotted sieve and placed in a baker of Teflon. (4.5 mL) and HNO3 (1.5 ml) concentrate and then heat on a heat plate at 80 ° C. Then add 4 mL of 1:1 mixture of perchlorate and hydrofluoric acid and then until near dehydration. Take the leachate and complete the volume with ions free distilled water to 25 ml, then centrifuge with 3000 cycles / min for 20 minutes and then transfer the solution to polyethylene bottles and store until the measurement of the Atomic Absorption Spectrophotometer (AA-7000)

Results and Discussion

Table (1) shows the type of company producing the fish species studied

<table>
<thead>
<tr>
<th>Type of fish</th>
<th>Product Country</th>
<th>company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canned tuna</td>
<td>Iran</td>
<td></td>
</tr>
<tr>
<td>Fishy frozen fish</td>
<td>Vietnam</td>
<td></td>
</tr>
</tbody>
</table>
Cont... Table (1) shows the type of company producing the fish species studied

<table>
<thead>
<tr>
<th>Fish Species</th>
<th>Company</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rao is frozen Minmar</td>
<td>Imported specifically for a good farms company for agricultural and livestock production and marketing</td>
<td></td>
</tr>
<tr>
<td>Stromateus Sp. frozen fish</td>
<td>Vietnam</td>
<td>Imported by Al-Muntar Company for General Trading and Contracting (Dolphin sign)</td>
</tr>
<tr>
<td>Canned sardines</td>
<td>Production and export of Sombraisha Trading Co. TAMAN-SIDO ARGO / INDONESIA</td>
<td>Importer Company Ideal - Haram - Giza (Egypt)</td>
</tr>
</tbody>
</table>

Table (2) Concentrations of heavy metals in Galsam and Zubaidi muscles in a unit (ppm)

<table>
<thead>
<tr>
<th>Fabric</th>
<th>Cu</th>
<th>Cd</th>
<th>Pb</th>
<th>Cr</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gills</td>
<td>0.039</td>
<td>0.833</td>
<td>11.30</td>
<td>0.021</td>
<td>2.161</td>
</tr>
<tr>
<td>Muscle</td>
<td>ND</td>
<td>0.800</td>
<td>10.99</td>
<td>0.110</td>
<td>0.474</td>
</tr>
<tr>
<td>The average</td>
<td>0.039</td>
<td>0.8165</td>
<td>11.145</td>
<td>0.0655</td>
<td>1.3175</td>
</tr>
</tbody>
</table>

Table (3) Concentrations of heavy metals in Galsam and frozen erythrocytes (ppm)

<table>
<thead>
<tr>
<th>Fabric</th>
<th>Cu</th>
<th>Cd</th>
<th>Pb</th>
<th>Cr</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gills</td>
<td>0.053</td>
<td>0.019</td>
<td>0.231</td>
<td>ND</td>
<td>1.589</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.115</td>
<td>0.043</td>
<td>0.473</td>
<td>0.100</td>
<td>0.929</td>
</tr>
<tr>
<td>The average</td>
<td>0.084</td>
<td>0.031</td>
<td>0.352</td>
<td>0.100</td>
<td>1.259</td>
</tr>
</tbody>
</table>

Table (4) Concentrations of heavy metals in Ghalsam and muscles of raho thickness (ppm)

<table>
<thead>
<tr>
<th>Fabric</th>
<th>Cu</th>
<th>Cd</th>
<th>Pb</th>
<th>Cr</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gills</td>
<td>ND</td>
<td>0.051</td>
<td>0.595</td>
<td>0.031</td>
<td>4.470</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.0234</td>
<td>0.012</td>
<td>1.444</td>
<td>0.149</td>
<td>0.549</td>
</tr>
<tr>
<td>The average</td>
<td>0.0234</td>
<td>0.477</td>
<td>1.019</td>
<td>0.09</td>
<td>2.509</td>
</tr>
</tbody>
</table>

Table (5) Concentrations of heavy elements in frozen fish muscles (ppm)

<table>
<thead>
<tr>
<th>Type of fish</th>
<th>Cu</th>
<th>Cd</th>
<th>Pb</th>
<th>Cr</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuna muscles</td>
<td>ND</td>
<td>0.915</td>
<td>13.18</td>
<td>0.031</td>
<td>0.624</td>
</tr>
<tr>
<td>Sardine muscles</td>
<td>0.028</td>
<td>0.040</td>
<td>0.744</td>
<td>0.041</td>
<td>0.400</td>
</tr>
</tbody>
</table>
Fish are secondary consumers by feeding on some phytoplankton and small aquatic animals. They represent an important food source, helping to understand the accumulation of trace elements and their functional behavior in the organs and levels of food. They can also be used as good biomarkers to assess the health of the ecosystem because they occupy different levels of food Karadede and Unlü, 2007). It is therefore an input to identify the contamination of trace elements in aquatic environments and to identify the type of elements prevailing in that environment and thus to determine the suitability of fish for human consumption Obasohan, 2008; (Dural, et.al., 2007).

In the following order:
Stromateus Sp. Fish: Galsam
Lymphatic fish: Galsm <muscles
RAHO Fish: GALASM

The results showed that the general concentration in fish and trace elements studied were as follows:

The general concentration of Stromateus Sp fish according to the elements was as follows:
  Cadmium component: Galsm <muscles
  Copper element: Galsm <muscles
  Element Chromium: Muscle
  Lead element: Galsm <muscles
  Zinc element: Galsm <muscles

As for the general concentration in the pigeon fish and elements were as follows:
  Cadmium component: Galsm muscles
  Copper element: Galsm muscles
  Element Chromium: Muscle
  Lead element: Galsm muscles
  Zinc element: Galsm muscles

As for the general concentration of raho fish according to the elements were as follows:
  Cadmium component: Galsm <muscles
  Copper element: Labeo rohita muscles
  Element Chromium: Muscle
  Lead element: Galsm muscles
  Zinc element: Galsm <muscles

In the sardine and tuna muscles, the concentrations were as follows:
  Cadmium component: tuna muscles> sardine muscles
  Copper element: Sardine muscles
  Chromium Element: Sardine muscles <Tuna muscles
  Lead Ingredient: Tuna muscles> Sardine muscles
  Zinc element: Tuna muscles> Sardine muscles

These changes in concentrations of trace elements are very similar in their descending sequence to their presence in the studied members of the studied species, especially in concentrations of all elements in Stromateus Sp studied fish.

As noted from the above sequence, the muscles and gazelles of the fish are ineffective for the accumulation of trace elements, except for the lead element in the Stromateus Sp muscle. The concentration of the lead element in the Stromateus Sp muscle is observed and this is consistent with Bevoets et al. (2009) Cadmium and lead in carp fish with concentrations above the ECP threshold for 2002 (ppm0.05); however, the concentration of cadmium in our study was within the permissible limits of the WHO (0.05 ppm), possibly because the musculoskeletal tissue of Stromateus Sp was high in fat.

Financial Disclosure: There is no financial disclosure.
Conflict of Interest: None to declare.
Ethical Clearance: All experimental protocols were approved under the College of Dentistry/ University of Baghdad, Iraq and all experiments were carried out in accordance with approved guidelines.

References
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7. Flessas C, Pinel AB, Campbell PG. Analysis of biotic and a biotic factors influencing the bioaccumulation of trace metal in Bithyno tantaculats (Mollusca Gastropod) in Lac. Louis Quebec-120 Annu aquatic toxicity workshop, (Canada) 1993;17-21,Oct., 80-95.


Histological Study of the Adrenal Gland of the Adult Female Rabbit (Oryctolagus Cuniculus)

Faiza Jabar Joda1, Ahmed Obaid Hossain2
1Assistant lecturer, Director General of Education Rusafa,
2Assistant prof. College of biotechnology/Al-Qasim Green Univ.

Abstract
The aim of this study was to investigate the structure of the female rabbit adrenal gland by light microscopic observations. For this purpose, ten adrenals were collected from five adult female rabbits. The tissues were processed for light microscopy. The weight of rabbit gave mean values of 2.04 kg and the mean values of long axis of left adrenal gland 15.46 mm and the diameter of right gave mean values 11.30 mm. The adrenal glands sit at the top of the kidneys, one on each side of the body, and have an inner core (known as the medulla) surrounded by an outer shell (known as the cortex). The adrenal cortex consists of three anatomic zones: the outer zona glomerulosa; the intermediate zona fasciculata, which secretes cortisol; and the inner zona reticularis. The adrenal medulla, lying in the center of the adrenal gland, is functionally related to the sympathetic nervous system and secretes the catecholamines epinephrine and norepinephrine in response to stress.

Key word: Adrenal gland, Rabbit, Oryctolagus Cuniculus

Introduction
Rodents (Rodentia), the largest order of placental mammals, comprise more than half of the mammals known at present. The rabbit that is the subject of this study is from the Hystricidae family, which constitutes a small group of the order Rodentia (1,2,3). The adrenal gland is composed of two distinct portions: an outer cortex (mesodermal origin) and an inner medulla (neuroectodermal origin) (4,5,6,7). The adrenal cortex is subdivided into three (5, 6,7) or four (8) distinct zones of epithelial cells. The outermost zone is called zona glomerulosa in ruminants, in human (5,9) and in a few rodents (6,10,11) and is formed of irregular clusters.

Materials and Method
In this study, ten adrenal glands were collected from five adult female rabbits (the average of weight 4.2 kg and the average age five months & ten days). Animals were anaesthetized with Pentothal (6 mg/kg) and adrenal specimens were taken. For light microscopy, 10% formaldehyde solutions were used to fix the tissue. After fixation, the specimens were rinsed with buffer; treated with ethyl alcohol and xylol and embedded in paraffin blocks. Sections (5-7 μm thick) were made by microtome (Leitz) and then stained with hematoxylin-cosin. Photomicrographs were taken with a camera (Nikon) attached to a microscope. Micrometric measurements and volume were carried out with ocular micrometer.

Results
Data obtained from micrometric measurement of adrenal glands of adult rabbit are shown in table 1. The weight of rabbit gave mean values of 2.04 kg and the weight of right and left adrenal gland gave mean values of 0.14 and 0.15 g respectively. The mean values of long axis of left adrenal gland 15.46 mm and the diameter of right gave mean values 11.30 mm. The paired adrenal glands were located to the cranio-medial of the kidneys and both right and left adrenals were flat (fig.1). Morphological data obtained from this study showed that the adrenal cortex can be divided into three zones according to differences in the arrangement of its cells; an outer zone, the glomerulosa; a middle zone, the fasciculate; and an inner zone, the reticularis. The zona fasciculate is the broadest of the three. The zona glomerulosa is a relatively narrow zone in which the arrangement of the cord is such that the cells are in arcuate groups (fig 2). Fig.3 showed The cells tend to be columnar and they have spherical nuclei. A few lipid
droplets may be found in the cytoplasm, but they are sparse in comparison with the droplets in the zona fasciculata. The zona fasciculata, the broadest zone, is composed of cell cords coursing parallel in one another in a radial direction toward the medulla (fig. 4). The cords are usually only one or two cells in width. The secretory cells are generally cuboidal or polyhedral in shape and they are sometimes binucleate. The nuclei appear more vesicular than those of the glomerulosa, with less dense chromatin. The cells are relatively large. Since the lipids are dissolved by the usual technical procedures, the cytoplasm has a spongy appearance (fig. 5).

The zona reticularis is composed of network of cell cords (fig. 6&7). The cells are generally smaller than those of the fasciculata and they relatively few lipid droplets in comparison with the fasciculata and the droplets vary greatly in size.

**Medulla.** The adrenal medulla was about one quarter of the area of the gland. The chromaffin cells in irregular clusters, and at the cortico-medullary boundary cortical cells were observed. Epinephrine-storing cells (E-cells) were more numerous and smaller than norepinephrine-storing cells (NE-cells). The majority were moderately electron-dense E granules, but some were highly electron-dense NE granules. Ganglion cells were rarely observed. Numerous central sinusoidal vessels were noticed in place of a large central venule.

Table 1: Showing weight of rabbit in gm, weight in gm and volume in cm³ of right (Rt) and left (L) adrenal gland of five adult Rabbit. Mean ± standard deviation

<table>
<thead>
<tr>
<th>Rabbit number</th>
<th>Weight of Rabbit (kg)</th>
<th>Weight of adrenal gland (g)</th>
<th>Diameter/ mm</th>
<th>Long axis/ mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>0.15 0.15</td>
<td>9.68</td>
<td>7.25</td>
</tr>
<tr>
<td>2</td>
<td>2.2</td>
<td>0.14 0.15</td>
<td>6.77</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>1.9</td>
<td>0.13 0.14</td>
<td>7.61</td>
<td>9.33</td>
</tr>
<tr>
<td>4</td>
<td>2.3</td>
<td>0.15 0.16</td>
<td>6.2</td>
<td>8.8</td>
</tr>
<tr>
<td>5</td>
<td>1.8</td>
<td>0.13 0.14</td>
<td>6.9</td>
<td>9.5</td>
</tr>
<tr>
<td>Mean+SD</td>
<td>2.04±0.089</td>
<td>0.14±0.01 0.15±0.017</td>
<td>7.43±0.59</td>
<td>8.77±0.32</td>
</tr>
</tbody>
</table>

Fig(1) site of adrenal glands of female rabbit
Fig(2) Light photograph of the zona glomerulosa of adrenal gland in the female rabbits (H&E 200x)

Fig(3) Light photograph of the zona glomerulosa of adrenal gland in the female rabbits note that the cells of this zona have an apparent arcuate arrangement (two headed arrow) (H&E 400x)

Fig(4) Light photograph of the zona fasciculate of adrenal gland in the female rabbits (H&E 200x)
Discussion

The adrenal cortex is composed of two\textsuperscript{12}, three\textsuperscript{13}, or four\textsuperscript{14} distinct areas of epithelial cells. The outermost is called zona glomerulosa in ruminants and primates\textsuperscript{15}, and in a few rodents\textsuperscript{(12,11)}. In most other domestic species including dogs, cats, horses, and pigs the parenchyma forms archades just beneath the capsule.

Some investigators\textsuperscript{(8,4,7,15,16)} have reported that the zona fasciculata is the widest zone of the cortex and has a foamy appearance caused by the presence of numerous lipid vacuoles\textsuperscript{(8,5,6,7)}. The zona fasciculata forms 60\% of the cortex\textsuperscript{16} or 70.9\% of the cortex in the rabbit\textsuperscript{15}. According to\textsuperscript{7} the zona fasciculata is 65\% of the gland. Results of this study indicate that the zona fasciculata appears foamy and forms 66.3\% of the cortex and 48.5\% of the gland.

\textsuperscript{9} identified that the adrenal medulla in goat is about a quarter of the gland. According to\textsuperscript{7} the adrenal medulla is 13\% of the human gland. Our results are similar to those reported in goat by\textsuperscript{9}.

Previous reports described that the adrenal medulla contains both types of chromaffin cells\textsuperscript{(19,18)}. Furthermore, at the cortico-medullary boundary, cortical cells were together with chromaffin cells\textsuperscript{(18,19)}. Epinephrine cells were more numerous, smaller and contained less electron-dense granules than norepinephrine cells\textsuperscript{(17,18,19,5,20)}.

\textsuperscript{21} identified that the adrenal medulla in goat is about a quarter of the gland. According to\textsuperscript{22} the adrenal medulla is 13\% of the human gland. Our results are similar to those reported in goat by\textsuperscript{23}.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of biotechnology/Al-Qasim Green University, Iraq and all experiments were carried out in accordance with approved guidelines.

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22. Ahmed O H. carbimazole and its effects on th’hroid grand of fenrare rabbits. In-dian J Forensic Medicine & Toxicology, 2019; 13(3).

The Effect of Physical Exercises According to Deny Foot to Improve Some Skillful and Muscular Abilities among Fencing Young Players

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²Assistant lecturer, University of Wasit - College of Physical Education and Sports Sciences,
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Abstract

The research problem is about the lack of previous studies concerning with deny foot for feet through using the modern technological devices that record and save the quantities of the feet strength, by using Bluetooth. The researchers noted weakness in some muscle abilities, and reduce the level of physical performance for fencing players. This study aims at preparing exercises according to Deny Foot, which was put in the feet of the players, in order to improve some skillful and muscular abilities among fencing young players as well as to recognize the effect of these exercises on improving some skillful and muscular abilities among fencing young players.

The researchers used the experimental method with the experimental design of two research groups (Equivalence), first experimental group and second experimental group. The research community of fencing competition has identified by choosing (10) players of Al-Jaish Sports Club for young people under the age of 17-18 years. The special exercises were applied for eight weeks and by three exercises units per week, the researchers used the Statistical Package for the Social Sciences (SPSS). The most important conclusions which the researchers reached that physical exercise have positively influenced on the development of some muscular abilities and skills for young fencing players. The researchers recommend the importance of investing modern devices and players in scientific colleges or specialized institutes and use them to provide a clear vision and information about the place and timelines.

Keywords: Physical Exercises, Deny Foot, Muscular Abilities

Introduction

The fencing sport is considered one of the sports that require special skillful abilities and special physical characteristics ¹, the most important characteristic is the muscular ability because of this characteristic collect force and speed together, which the fencer depend on it directly, so the muscles ability such as which found in legs, arms shoulder and the torso has an important role² for the fencer not only in moving sword but it also has an important in fencer transfer speed of the fencer’s chest, which rushes forward and then bounces back as the fencer performs a movement attack to make a point against the competitor or return to the standby mode to To avoid the competitor’s movement ³. The requirement to do good achievement in this efficiency is to apply modern exercise methods, based on actual experimentation, those methods aimed at improving, components of achievement in fencing ⁴. From this stems, the importance of this study is to study the effect of exercise, in accordance with the magnitudes of the forces exerted, for step one, time using, a mobile device (Deny foot) in order to develop their own exercises to develop this contest, and pressure, level of performance, which reflects, the ability of the players of the physical ⁵ effort that is directly related to the performance development level and improving the skillful level in this competition according to scientific basis which linked with the components of its actual exercise, and thus keep up with the internationally advanced level of achievement.
Aims of the Study

This study aims at:

1. Making several exercises to improve some skillful and muscular abilities among fencing young players.
2. Identifying the effect of improving some skillful and muscular abilities among fencing young players.

Hypothesis

The researcher hypothesized the following: The exercises have positive effects in improving some skillful and muscular abilities among fencing young players.

Methodology

The researchers used the experimental method with the experimental design of two equivalence groups, first experimental group and second experimental group.

Table (1): Shows the Special Measurements of Research Sample in order to Similarity

<table>
<thead>
<tr>
<th>No.</th>
<th>Measurements</th>
<th>Units of Measurement</th>
<th>Cm.</th>
<th>Mean X</th>
<th>Torsion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age</td>
<td>Year</td>
<td>16.4</td>
<td>0.45</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>Training age</td>
<td>Year</td>
<td>6.39</td>
<td>3.23</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>Mass</td>
<td>Kg.</td>
<td>66.3</td>
<td>3.5</td>
<td>70</td>
</tr>
<tr>
<td>4</td>
<td>Length</td>
<td>M.</td>
<td>1.63</td>
<td>0.08</td>
<td>1.66</td>
</tr>
</tbody>
</table>

Table (1) shows that Torsion is null for all the values, which refers to the normal distribution which means all the members of the research sample is similarity.

Table (2): Illustrates the Equivalence by Using T-Test for the Dependent Variables of the Research Sample

<table>
<thead>
<tr>
<th>No.</th>
<th>Statistical Process Research Variables</th>
<th>Units of measurement</th>
<th>The first experimental group</th>
<th>The second experimental group</th>
<th>T-test value</th>
<th>Significant difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Wide jump test, stability</td>
<td>Cm.</td>
<td>173.5</td>
<td>20.42</td>
<td>179</td>
<td>15.85</td>
</tr>
<tr>
<td>2</td>
<td>Test of medical ball throwing, sitting</td>
<td>Cm.</td>
<td>3.43</td>
<td>0.22</td>
<td>3.47</td>
<td>0.46</td>
</tr>
<tr>
<td>3</td>
<td>Test of the speed movement of feet (headway)</td>
<td>Sec.</td>
<td>4.21</td>
<td>0.55</td>
<td>4.13</td>
<td>0.52</td>
</tr>
<tr>
<td>4</td>
<td>Test of the speed movement of the feet (fall back)</td>
<td>Sec.</td>
<td>4.87</td>
<td>0.47</td>
<td>4.88</td>
<td>0.84</td>
</tr>
</tbody>
</table>

- At freedom degree (FD): (10-2=8) with a significance level (0.05).
Practical Framework:

Measuring System of Deny Foot

It is a system to measure the variables of deny foot on ground through every step during running, which is made up of four parts that is considered as a base to conduct data (which is considered as foot place that shoes placed in, with a connection wire for the computer which connected with the experimenter), as well as a signal receiver that linked with a laptop and receiving signals for 30 m., the system works when the experimenter put the device on his/her foot put it in the leg with the sensor steps on the experimenter’s foot, then entering the data about the experimenter’s age, weight, length and sex, the system measures the variables of speed and recorded space of every step, these data may be used to food the training units to save information in the system.


The aim of the test is to measure the muscular ability of the feet, in the forward start up.

The Used Instruments: an appropriate place for starting up with (1.5 m.) *(3.5 m.), the place must be standard and free of chalk.

The Performance Description: The experimenter stands behind the beginning line, the instep must be on the outside of beginning line. The experimenter begins to swing the arms to back with the knees bent and tilt to forward a little, and then jumps forward to the maximum by lying the knees up and pushing the feet then swing arms ahead.

Measuring points: The measurement is from the starting line to the last part of the body touching the ground. Three attempts are given to the experimenter. The experimenter is credited with the best try.

The Test of Depth Jump Height

The aim this test is to measure the muscular ability of feet.

Instruments: Board put in the wall that its bottom edge must be about 150 cm. from the ground, then it must be put in subsequently (151 to 400 cm.), chalk toe (for the purpose of marking the board). The board can be dispensed with, by putting the marks on the wall according to the performance conditions. The height of the box is 45 cm.

The Performance Characteristics: The experimenter floods the distinctive fingers of the hand of chalk prepared for it, then jumped using a box where the player stands on the box and then down to the ground trying to jump to the top instantaneously and mark the distinctive hand, where the experimenter rises with the feet with vertical jumping and touching one hand, every experimenter has three attempts to score his/her best.

Recording Way: the first mark is placed before jumping (by extending the arms high from the ground and a first mark facing the wall). Then a second mark of jumping (after landing from the box and jumping high) and the distance between the two markers, reflects the degree of experimenter testing (jump distance).

Physical Tests Including:

The sped of moving feet tests (starting up): (Yassir Muhamed Ahmed Hajar, 2007: 43-44).

The aim of the test: To measure the speed of performance progress distance of 14 meters.

Instruments used: Duct tape - 2 clocks (timers) – fencing stadium divided into two parts.

Method of doing the test: From standby position the experimenter stands on the starting line specified on the stadium so that the performance of every 2 experimenters together for the element of competition in performance when hearing the signal occupies the clock, the player progresses at full speed forward to reach the finish line and when touching the back foot the finish line closes the clock.

Performance conditions:

- The test is conducted among the players, every player in a section.
- Maintaining a standby position during the test performance.
- Perform moving forward correctly.
- Going in a straight line.
- Turn off the clock after the back foot touches the line.

Recording Way: The time is calculated for the nearest 1/100 sec., gives three tries and depended on the
best one.

*Test the speed of movements of the feet (regression) (Ibid: 44-45)

The aim of the test: measuring the speed of the performance of the regression distance of 14 meters.

Instruments used: Duct tape - 2 clocks (timers) - fencing stadium divided into two parts.

Method of doing the test: From standby position the experimenter stands on the starting line specified on the stadium so that the performance of every 2 experimenters together for the element of competition in performance when hearing the signal occupies the clock, the player progresses at full speed forward to reach the finish line and when touching the back foot the finish line closes the clock.

Performance conditions:
- The experimenter stands of the wall and in front of fencing stadium.
- The test is conducted between two experimenters and every player on his or her section.
- Continue standby during testing.
- Perform backwards correctly.
- Backwards in a straight line.
- Turn off the clock after the back foot touches the line.

Recording Way: The time is calculated for the nearest 1/100 sec., gives three tries and depended on the best one.

**Pre-Tests:**


**The Main Experience**

- Researchers have prepared special real-time exercises in pre tests.
- The exercises were given three training units per week on days (Saturday, Monday and Wednesday).
- The experience started on Saturday, March 10, 2019 and ended on Thursday, May 9, 2019.
- The period of the exercises was within the period of the special numbers and by 6 weeks and thus 24 exercise units were applied.

**Post-Tests:**

The researcher conducted the post-tests at al-Jaish Sports Club Stadium on Saturday, 11/5/2011 until Sunday, 12/5/2019 (after the completion of the proposed exercises and the same steps and the conditions under which the tests were conducted)

**Statistical Devices**

The researchers used the Statistical Package for the Social Sciences (SPSS).

**Results and Discussion**

presentation, analysis and discussion of the results of physical tests for the first and second experimental groups:

**Table (3) shows the total difference and the total difference box and the average difference of computational circles and the standard error of the difference of computational circles between the pre and post-tests and the calculated and tabulated (t) value of the first and second experimental groups for physical tests.**

<table>
<thead>
<tr>
<th>Test name</th>
<th>Statistics Groups</th>
<th>Calculated T-value</th>
<th>Sig. Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wide jump stability (Cm.)</td>
<td>1st experimental</td>
<td>207</td>
<td>3471.5</td>
</tr>
<tr>
<td></td>
<td>2nd experimental</td>
<td>160</td>
<td>2577.3</td>
</tr>
<tr>
<td>High deep jump (cm.)</td>
<td>1st experimental</td>
<td>10</td>
<td>62.84</td>
</tr>
<tr>
<td></td>
<td>2nd experimental</td>
<td>21</td>
<td>53.5</td>
</tr>
</tbody>
</table>

* At FD (5-1) = 4 with Sig. level 0.05.
Presentation and analysis of the results of the physical tests of the first and second experimental groups of the post-test:

Table (4) shows the Arithmetic Mean (X) and Standard Deviation (SD) of the physical tests (Wide jump stability and High deep jump) of the two experimental groups and the calculated T–value of the post-test

<table>
<thead>
<tr>
<th>Statistics Test</th>
<th>The first experimental group</th>
<th>The second experimental group</th>
<th>Calculated T-value</th>
<th>Sig. differences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Wide jump stability (Cm.)</td>
<td>208</td>
<td>25.7</td>
<td>23.5</td>
<td>6.04</td>
</tr>
<tr>
<td>High deep jump (cm.)</td>
<td>38.5</td>
<td>5.75</td>
<td>36</td>
<td>4.46</td>
</tr>
</tbody>
</table>

At FD (10-2) = 8 with Sig. level 0.05.

Discussion the Results of the Physical Tests:

By observing the table (3) we find that all the tests are moral between the pre and post-test of the first and second experimental group and attribute this to the effect of using a device sensitive force of the feet and adjust the amounts of force exerted for one step.

The implementation of these exercises in the period of special preparation has also contributed to the development of some physical abilities to the importance of skill exercise and the development of muscle strength in the period of special physical preparation, as the strength must be made in different forms and amounts and to correspond accurately with the performance of skills as the development of that strength depends on secret changes, the rhythm of nerve signals as the muscular activity is characterized by a high degree of compatibility between the time and size of the nerve signals, and indicates the importance of this special force through performance exercises which is the main means of its development (Mamdouh Muhammad Al-Shanawi, 2003: 112)

Khairya Ibrahim Al-Sukkari and Muhammad Jabir state that when performing exercises for certain muscle groups, it results in adjustments in specific muscle areas, for example, the effectiveness of endurance can be increased only by endurance exercises, i.e. when performing specific exercises that produce specific adaptations, if you want to jump up or forward, the content of the exercise must base on jumping exercises and if you want speed you have to focus on speed exercise and so on... It is imperative to develop exercise programs according to the type of activity practiced (Khairya Ibrahim Al-Sukkari and Muhammad Jabir, 2005: 25).

Present, analyze and discuss the results of the skill tests for the first and second experimental groups:

Presentation and analyzing of the results of the skill tests for the first and the second experimental groups
Table (5) shows the total difference, the total difference square, the average difference of the computational circles, the standard error of the computational circles difference between the pre and post-tests and the calculated (t) value of the first and second experimental groups of the skill tests.

<table>
<thead>
<tr>
<th>Test name</th>
<th>Statistics Groups</th>
<th>Calculated T-value</th>
<th>Sig. Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed of feet movement starting up (sec.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st experimental</td>
<td>2.86</td>
<td>0.6278</td>
<td>0.48</td>
</tr>
<tr>
<td>2nd experimental</td>
<td>1.28</td>
<td>0.3064</td>
<td>0.21</td>
</tr>
<tr>
<td>Speed of feet movement backing (sec.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st experimental</td>
<td>3.1</td>
<td>0.7764</td>
<td>0.52</td>
</tr>
<tr>
<td>2nd experimental</td>
<td>2.78</td>
<td>1.547</td>
<td>0.46</td>
</tr>
</tbody>
</table>

* At FD (6-1) = 5 with Sig. level 0.05.

Presentation and analyzing of the results of the skill tests for the first and the second experimental groups of the post-test

Table (6) Shows the Arithmetic Mean (X) and Standard Deviation (SD) of the skillful tests (Test of the speed movement of feet- headway, Test of the speed movement of the feet- fall back) of the two experimental groups and the calculated T–value of the post-test

<table>
<thead>
<tr>
<th>Statistics Test</th>
<th>The first experimental group</th>
<th>The second experimental group</th>
<th>Calculated T-value</th>
<th>Sig. differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test of the speed movement of feet, headway (Sec.)</td>
<td>3.92 0.23</td>
<td>3.71 0.53</td>
<td>0.001</td>
<td>Sig.</td>
</tr>
<tr>
<td>Test of the speed movement of the feet, fall back (Sec.)</td>
<td>4.37 0.076</td>
<td>4.34 0.49</td>
<td>0.003</td>
<td>Sig.</td>
</tr>
</tbody>
</table>

* At FD (10-2) = 8 with Sig. level 0.05.

The plyometric exercises contributed the improving the movements of feet, Talha Hussam al-Din indicates that the exercises of the plyometric used have an effect on the central nervous system by reducing decelerating processes and recruiting muscle fibers to do the muscle work characterized by the strength of the contraction and its speed (Talha Hussam al-Din, 1999: 95).

Conclusions

The researchers found out the following conclusions:

1. The suggested exercises have positively effected in some physical and skillful abilities of Fencing Young experimenters.

2. Using modern technical instruments to identify the number of the correct steps in each second as well as using it as an instrument to observe the performance speed.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols
were approved under the Middle Technical University, Iraq and all experiments were carried out in accordance with approved guidelines.

References

Assessment of Primary School Teachers' Knowledge Towards Post-Traumatic Stress Disorder among Pupils In Mosul City

Nawaf Mohammed Dhahir1*, and Saja Hashem Mohammed2.
1Ph.D /Instructor, Faculty of Nursing, University of Babylon, Iraq, 2Professor, Faculty of Nursing, University of Babylon, Iraq.

Abstract

Background: Teachers’ knowledge towards childhood mental health disorders at schools located in regions which were exposed to armed conflict, traumatic events and wars should be given the highest priority, not only for pupils’ mental health, but also as an important perspective for education. Therefore, teachers' knowledge must be adequately assessed about common childhood mental health disorders, particularly post-traumatic stress disorder (PTSD).

Objectives: The study aimed to assess teachers' knowledge towards post-traumatic stress disorder (PTSD) among pupils at primary schools in Mosul city.

Materials and Method: A descriptive study was conducted for the period from 1st December 2018 to 30th May 2019. The study included (520) primary schools inside Mosul which is the second largest city in Iraq. A probability sampling (simple random technique) was used, the sample consisted of (60) teachers who were teaching in primary schools inside Mosul city. Data were collected through the utilization of constructed questionnaire after validity and reliability of it were estimated. Reliability of the questionnaire was estimated through a pilot study and the validity was determined through (15) experts related to the field of study. All data were collected by means of interview technique. Data were analyzed through use of descriptive and inferential statistical analysis procedures using SPSS Version-23.

Results: The findings of current study indicates that the level of teachers' knowledge towards post-traumatic stress disorder (PTSD) was low.

Conclusion: The study concludes that there is a considerable lack in teachers' knowledge in regard to post-traumatic stress disorder (PTSD).

Keywords: Assessment, Post-Traumatic Stress Disorder, Teachers' Knowledge.

Introduction

Teachers have an important role in supporting pupils when they feel fear, anxiety and/or sadness as well as in referral these pupils to mental health professionals and facilities. Pupils, especially those at schools located in regions which were witnessed armed conflict, traumatic events and wars, are more susceptible to experience psychological distress symptoms than others. In order to enhance teachers' roles, their knowledge about common childhood mental health disorders should be assessed effectively, particularly post-traumatic stress disorder (PTSD) as one of the common mental health disorders among pupils in Mosul city [1]. In Iraq, Mosul city witnessed a numbers of wars and conflicts since 2005. The last conflict was triggered by the invasion of the criminal (ISIS) gangs in the beginning of 2014. As a result, the experience of war and violence increases the risk for emotional disturbing and development of psychiatric disorders among individuals. It is obvious that traumatic experiences affect people, especially children, in different ways. Posttraumatic Stress Disorder (PTSD) is a disorder that some people experience after living through a stressful and traumatic events. Traumatic events may include combat, severe accidents, or physical assault [2, 3]. Childhood trauma can have a direct and considerable impacts on the ability of a pupil...
to learn. Yet, this issue has largely been neglected by our education system [3]. Adopting a trauma-informed approach, could enable schools to conduct a model shift at the teachers and other staff to recognize, make referral, understand and address mental health and learning needs of traumatized pupils. This requires a commitment to shaping school practices and policies to be sensitive to the needs of traumatized learners. This effort positively affects schools and changes the life-track of vulnerable pupils[4]. Poor emotional and mental health impacts a considerable number of children and young people[5]. Teachers are in a unique position to identify pupils who are experiencing difficulties after stressful events due to their prolonged and closed contact with pupils[6]. Following a traumatic event, children may respond in different range of responses, so it isn't obviously what types of reactions they will show. Therefore, building strategies to help teachers in winding up additional more attuned for identifying the symptoms of emotional and behavioral difficulties in their pupils following a stressful events is very necessary[7, 8].

Objectives: The study aimed to assess teachers' knowledge regarding post-traumatic stress disorder (PTSD) among pupils at primary schools in Mosul city.

Methodology

A descriptive design using the assessment approach was conducted for the period from 1st December 2018 to 30th May 2019 to assess the teachers' knowledge toward post-traumatic stress disorder (PTSD) among pupils at primary schools in Mosul city. A probability sampling (simple random technique) was used. A sample of (60) primary schools were selected after dividing the city into two sides (Right and left side of the Tigris River), then selected (30) schools from each side and then one teacher was selected from each school in a probable manner. The teachers were participated voluntarily in the study. The data were collected through using a special questionnaire after the validity and reliability of it were estimated. The content validity of the questionnaire was estimated through a panel of experts related to the study field, and its reliability was estimated through a pilot study which included (10) teachers who were excluded from the final sample. The reliability of study instrument was determined by using test-retest technique, the alpha correlation coefficient (r) was =0.82. The questionnaire was constructed by the researchers after reviewing many related literatures, it is composed of two main parts: Part-I: Teachers' socio-demographical data such as (age, gender, marital status, years of employment, and educational level). Part-II: This part is concerned with multiple choice questions (MCQs) to assess teachers' post-traumatic stress related knowledge which included six sections as follow:

1- Section one: Eight (MCQs) about concept of health and childhood mental health.

2- Section two: Eight (MCQs) about the psychological trauma.

3- Section three: Eight (MCQs) about the impacts of psychological trauma.

4- Section four: Eight (MCQs) about Post-traumatic Stress Disorder symptoms.

5- Section five: Eight (MCQs) about DSM-5 Criteria for PTSD.

6- Section six: Eight (MCQs) about protection and resilience factors.

Cut of points for scores for each section: Extremely Low= (0-1), low= (2-3), Intermediate= (4-5), Good= (6-8). Cut of points for scores for total knowledge: Extremely Low= (0-12), low= (13-24), Intermediate= (25-36), Good= (37-48).

Results

Table (1): Socio-demographical Characteristics of Study Subjects (n=60):

<table>
<thead>
<tr>
<th>Demographical Characteristics</th>
<th>Groups</th>
<th>Sample (n = 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-40 years</td>
<td>32</td>
<td>53.4</td>
</tr>
<tr>
<td>41-50 years</td>
<td>17</td>
<td>28.3</td>
</tr>
<tr>
<td>51-60 years</td>
<td>11</td>
<td>18.3</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>42.40 (7.66)</td>
<td></td>
</tr>
</tbody>
</table>
This table indicates that all participants of the study are (60) teachers. Their ages are between (30-60) years old. The overall mean age for the participants is [42.40 (SD=7.66)]. The table also reveals that the highest percentage of the sample (53.4%) are in the age group of (30-40) years old, while the lowest percentage of them (18.3%) are at the age group of (51-60) years old. The table also shows that about (51.7%) of the sample are male. On the other hand, the majority of teachers (65%) are married. Regarding teacher’s work experience, the table indicates that (35%; n=21) of the sample are having years of employment ranged between (11-15) years. Finally, the table demonstrates that about (38.3%) of sample are possess an institute degree certificate and approximately (31.7%) are having university degree certificate.

**Table(2): Distribution of Study Subjects among Post-traumatic Stress Disorder Related Knowledge Sections according to their Levels of Knowledge:**

<table>
<thead>
<tr>
<th>Knowledge Sections</th>
<th>Extremely Low</th>
<th>Low</th>
<th>Intermediate</th>
<th>Good</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>%</td>
<td>F</td>
<td>%</td>
<td>F</td>
</tr>
<tr>
<td>Section One</td>
<td>1</td>
<td>1.7</td>
<td>29</td>
<td>48.3</td>
<td>28</td>
</tr>
<tr>
<td>Section Two</td>
<td>4</td>
<td>6.7</td>
<td>34</td>
<td>56.7</td>
<td>21</td>
</tr>
<tr>
<td>Section Three</td>
<td>3</td>
<td>5.0</td>
<td>39</td>
<td>65.0</td>
<td>17</td>
</tr>
<tr>
<td>Section Four</td>
<td>5</td>
<td>8.3</td>
<td>38</td>
<td>63.3</td>
<td>16</td>
</tr>
<tr>
<td>Section Five</td>
<td>6</td>
<td>10.0</td>
<td>29</td>
<td>48.3</td>
<td>24</td>
</tr>
<tr>
<td>Section Six</td>
<td>2</td>
<td>3.3</td>
<td>30</td>
<td>50.0</td>
<td>27</td>
</tr>
<tr>
<td>Total Knowledge</td>
<td>3</td>
<td>5.0</td>
<td>52</td>
<td>86.7</td>
<td>5</td>
</tr>
</tbody>
</table>

F: Frequency, %: Percent.
This table indicates that the highest percentage of teachers are having low levels of post-traumatic stress disorder related knowledge scores in all sections; section one (F= 48.3%; n= 29); section two (F= 56.7%; n= 34); section three (F= 65%; n= 39); section four (F= 63.3%; n= 38); section five (F=48.3; n= 29); section six (F=50%; n= 30). Concerning the levels of the total knowledge scores, this table shows that the highest percentage of teachers are having low level of knowledge (F= 86.7%; n= 52).

**Discussion**

The limitation that the researchers faced was that there was no related literature and previous studies about such assessment regarding post-traumatic stress disorder among pupils. This study objected to assess teachers’ knowledge in respect to childhood post-traumatic stress disorder (PTSD) at primary schools.

Data analysis revealed that (51.7%) of the sample are male, table (1). Relative to age, the study showed that the highest percentage of the sample (53.4%) are in the age group of (30-40) years old, the overall mean age for the participants is 42.40 (SD=7.66), table [1]. Regarding years of employment, the findings indicated that (35%; n=21) of the sample are having years of employment ranged between (11-15).

In the other hand, the study illustrated that the majority of teachers (65%) are married. Finally, the results demonstrated that about (38.3%) of sample are possess an institute degree certificate and approximately (31.7%) are having bachelor’s degree certificate, table (1).

These results are relatively in agreement with the findings of a previous study done by Al-kraawi (2018). Al-kraawi was applied an education program on primary school teachers regarding their knowledge about Attention deficit-hyperactivity disorder among pupils at primary schools in AL- Najaf city. Al-kraawi assessed teachers’ knowledge toward Attention deficit-hyperactivity disorder among pupils at primary schools. In his mentioned study, Al-kraawi proved that there is a significant lack in teachers’ knowledge towards such common childhood mental health disorder among pupils.

This current study also illustrated that the highest percentage of teachers are having low levels of PTSD related knowledge in all sections, table (2); section one (F= 48.3%; n= 29); section two (F= 56.7%; n= 34); section three (F= 65%; n= 39); section four (F= 63.3%; n= 38); section five (F=48.3; n= 29); section six (F=50%; n= 30). Concerning the levels of the total knowledge scores, this table shows that the highest percentage of teachers are having low level of knowledge (F= 86.7%; n= 52).

Finally, table (2) showed that the total knowledge scores of all post-traumatic stress disorder (PTSD) sections is low among (86.7%) of teachers who participated in the study. Therefore, as results mentioned above, we can say that there is a significant lack in teachers’ knowledge towards childhood post-traumatic stress disorder (PTSD).

**Conclusions**

The study concluded that there is a significant lack in teachers’ knowledge in regard to post-traumatic stress disorder (PTSD).

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Faculty of Nursing, University of Babylon, Iraq and all experiments were carried out in accordance with approved guidelines.

**References**


9. Al-kraawi MH. Effectiveness of an education Program on primary school Teachers’ Knowledge about Attention deficit-hyperactivity disorder among pupils in AL- Najaf city, Unpublished dissertation, Faculty of nursing, University of Babylon. 2018; 85.
Predictors of Bone Mass Density in Patients with Chronic Obstructive Pulmonary Disease

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Abstract

Background and Aim: The low bone mass as presented as osteopenia and osteoporosis represent example of the morbidities that occur in patients with chronic obstructive pulmonary disease (COPD). Predictors of bone mass density include osteopenia and osteoporosis. There is little data from Iraq on the relation between COPD and low bone mass. This study aimed to assess predictors of bone mass density in COPD patients attending Merjan Medical City.

Patients and Methods: The study was performed on patients with COPD patients seen at the Merjan Medical City in the period between January 2018 and June 2019. The study included taking history with physical examination in addition to the needed investigation which included spirometry and dual-energy X-ray absorptiometry (DEXA) scan, osteopenia was defined if the T score was -<1 to <-2.5 and osteoporosis of <-2.5 as according to the WHO guidelines.

Results: The study involved 122 patients with COPD, average age was 61.52±10.01, males were 107 (88.52%) and females were 15 (11.48%), 22 patients (18.03%) had normal bone mass while the remaining 100 (81.97%) had with low bone mass, 64 (52.46%) were osteoporotic and 36 patients (29.51%) had osteopenia. There was also significant relation between T score and FEV1, (P value= 0.000), significant relation between T score and smoking (P value 0.000), in addition there was significant relation between pack year and severity of airways obstruction as assessed by pulmonary function test (P value 0.000).

Conclusions: Our study shows that patients with low bone mass that presented as osteopenia and osteoporosis form high percentage in patients with COPD and greatly related to disease severity and amount of smoking.

Keywords: Chronic obstructive pulmonary disease, osteopenia, osteoporosis.

Introduction

Chronic obstructive pulmonary disease (COPD) is considered as a pulmonary long-standing debilitating disease that has many systemic manifestations. Predictors of bone mineral density include osteoporosis and osteopenia that can be diagnosed by using DEXA scan. Osteopenia, osteoporosis and fragility fractures are examples of the serious systemic manifestations that occurs in COPD patients due to low bone mineral density, in osteoporosis the T-score is below -2.5 while in osteopenia the T-score is between -1.0 and -2.5. The risk factors for causing decrease in bone mass in COPD are age, gender (mostly females), low exercise levels, impaired nutritional status, tobacco smoking and corticosteroids use. The dual-energy X-ray absorptiometry (DEXA) method is the gold standard for measuring bone mineral density (BMD). Many studies showed that osteoporosis and osteopenia commonly occur in patients with COPD and the prevalence in the range of 24-44% is reported. Osteoporosis usually were overlooked and remain undertreated which may be due to that it is a silent disease until it manifests clinically as pathologic fracture. Therefore, the primary focus of physicians in patients with COPD is to improve and maintain lung function without realizing that these patients suffer from low bone mass, which in turn increases the risk of fragility fractures and this could lead to an increase in pain and a worsening of respiratory function. There are few studies about the relation between...
COPD and BMD in Iraq \(^{11-13}\). This study aimed to assess BMD in COPD patients who attended Merjan Medical City and were investigated with a DEXA scan to assess bone mass.

**Patients and methods**

This is a cross-sectional study that was done in Merjan Medical City in the period from November 2018 to July 2019, the study included 120 patients (107 males and 15 females) suffered from COPD.

Demographic data of the patients were taken which included ages of patients, gender, height and weight for calculating body mass index (BMI), duration of the COPD smoking status as assessed by pack years, DEXA scan to assess bone mineral density. Pulmonary function tests were measured by using the computerized spirometer (MIR company, Italy). The forced expiratory volume in first second (FEV1) is a reliable and valid test to assess lung function according to standards of the American Thoracic Society \(^{14}\). The level of COPD severity was determined by The FEV1\% predicted depending on the global initiative of obstructive lung disease (GOLD) \(^{15}\).

**Data analysis**

The Statistical Package for Social Sciences (SPSS) (version 22) was used to analyze the data of this work. Results were presented as means ± standard deviation. Cross tabulation was used to compare between variables. The level of statistical significance was P-values < 0.05.

**Results**

The study involved 120 COPD patients, the mean age was 61.52±10.01 as shown in table (1).

**Table (1): Demographic data**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>24.00</td>
<td>82.00</td>
<td>61.5246</td>
<td>10.01917</td>
</tr>
<tr>
<td>BMI</td>
<td>14.00</td>
<td>34.00</td>
<td>22.4672</td>
<td>5.04544</td>
</tr>
<tr>
<td>FEV1</td>
<td>21.00</td>
<td>68.00</td>
<td>45.2623</td>
<td>14.28159</td>
</tr>
<tr>
<td>Pack year</td>
<td>15.00</td>
<td>100.00</td>
<td>54.3934</td>
<td>25.77928</td>
</tr>
</tbody>
</table>

Distribution of smoking status was illustrated in table 2, the percentage of active smokers was 54% while that of ex-smokers was 46%.

**Table (2): Distribution of smoking status**

<table>
<thead>
<tr>
<th>State</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active smoking</td>
<td>66 (54)</td>
</tr>
<tr>
<td>Ex-smoking</td>
<td>56 (46)</td>
</tr>
<tr>
<td>Total</td>
<td>122 (100.0)</td>
</tr>
</tbody>
</table>

The study showed that the percentage of osteoporosis was 52.46% while osteopenia was 29.52 % as illustrated in figure (1).
Figure (1): distribution of low bone mass in COPD patients

Figure (2) shows the relation between bone mineral density and BMI, osteoporosis was high in patients with BMI <19 while in normal subjects most of them was in 19-24 BMI, and the relation was significant (p value =0.000)

![Bar Chart](image)

**Fig (2) Relation between T score and BMI**

The study also showed significant relation between T score and FEV1, (P value= 0.000) as illustrated in figure (3)

![Bar Chart](image)

**Fig (3) Relation between T score and severity of pulmonary function**

There was significant relation between T score and smoking (P value= 0.000), as shown in figure (4)
Discussion

The study showed that the percentage of osteoporosis was 52.46% while osteopenia was 29.52% which means that about 82% of our patients had low bone mass and this was slightly similar to a study performed in Saudi Arabia where the percentage of low bone mass was 90% (16). The study also showed that patients who were suffering from osteoporosis were older, had longer COPD duration and lower FEV1. In a study performed by Graat-Verboom et al. (2009) in a review of 13 studies that involved 775 COPD patients, they reported that the prevalence of osteoporosis in COPD varies between 9 and 69% and that of osteopenia between 27% and 67%.

The study revealed significant relation between low bone mass and severity of airways obstruction and there was 26% of patients who had very severe COPD (GOLD stage IV) had osteoporosis and 9.8% in patients who had moderate COPD (GOLD stage II) and this agreed with a study by Alsayad et al. from south region of Saudi Arabia that found that the percentage of osteoporosis was 21.4% (16), while in a study by Tschopp et al. (2002) found 69% of patients with GOLD Stage II had osteoporosis whereas Bolton et al. (2008) found that 20% of patients with GOLD stage II had osteoporosis.

The study also showed significant relation between pack year and T score and the effect was dose-dependent that means increased risk of osteoporosis with increased amount of smoking. There are multiple causes behind this effect. Smoking has adverse effects on enzymes and hormones involved in bone regulation like parathyroid hormone and alkaline phosphatase, it is also a risk factor for ischemic osteonecrosis which is associated with nicotine’s peripheral vasoconstrictor effects.

The study also revealed significant relation between T-score and smoking status.

Conclusion

We conclude from this study that the low bone mass is common in COPD patients which have a relation with the disease severity and smoking. Bone mass index should be investigated in every patient with COPD by DEXA scan in order to be treated accordingly.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Medicine and all experiments were carried out in accordance with approved guidelines.

References


Quality Assessment of Commercial Fruit Juices and Health Hazards in Slemani City- Kurdistan Region/ Iraq

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Abstract

Fruit juices are especially valued in the human diet as these contain micronutrients, fiber, potassium, vitamin C, which work as antioxidants within the body as well as bio-functional components. The goal of this study is to determine the levels of Iron Fe, Zinc Zn, Magnesium Mg, Sodium Na, Potassium K, Calcium Ca and heavy metal manganese Mn, mercury Hg, Lead Pb as fruit juice consumed largely and will be in raise daily. Sixty-nine samples of different fruit juice were bought from retail markets in Sulaimani city, were analyzed using wet methods. The results obtained showed the ranges of the metals detected in all the of fruit juice were; Iron Fe (0-2), Zinc Zn (0 ), Manganese Mn (0), magnesium Mg (0-0.6), Sodium Na (0-22.9), Potassium K (0-19.2), Calcium Ca (0-29) and heavy metal mercury Hg (0-0.25), Lead Pb (0-4) mg/L. The mean value of Iron Fe, Magnesium Mg, Sodium Na, Potassium K, Calcium Ca and heavy metal mercury Hg, Lead Pb 0.421 , 0.16 , 4.188 , 3.348 , 7.362 ,0.029 , 0.578mg/L) respectively, Zinc and Manganese were not detected in any samples,some of the samples have nutrient value for sodium, potassium and calcium.

Keyword: Heavy metals, fruit juice, public health, flame photometer, toxic metal.

Introduction

Fruit juices are especially valued in the human diet as these contain micronutrients, fiber, potassium, vitamin C, which work as antioxidants within the body as well as bio-functional components (32) carotenoids, minerals (especially Mg, K) and various kinds of antioxidants and dietary fiber (pectin) is protective against degenerative and chronic diseases such as cancer and cardiovascular diseases 8,20. Fruit juice is commonly consumed by our youths not for their nutritional benefits, but to quench thirst during the hot summer, or maybe they think that these soft drinks will help them in digestion, while athletes use energy drinks to keep up their energy during intense physical activity and competitions. The market for these beverages has increased in the past years and although they might be harmless, overdoses or a combination of these with other drinks could be harmful to the health of some consumers in certain circumstances 19. Fruit juices are regularly utilized for culinary and dietary purposes 10. They are made up of chiefly cellulose, heme-cellulose and pectin substances that give them their texture and firmness. Fresh fruits and vegetables are of great importance in the diet because of the presence of vitamins and mineral salts 10. In addition, they contain water, calcium, iron, sulphur and potash 21. They are very important protective foods and quite useful for the maintenance of health and the prevention and treatment of various diseases 7. At higher concentrations, they may be toxic to the biota and could disturb the biochemical process and cause hazards 1. Heavy metal composition of food is of interest because of their essential and toxic nature, for example Fe, Zn, Cu, Cr, Co & Mn are essential while Pb, Cd, Ni & Hg are toxic at certain levels 16. Iron fortification in foods has been increased to tackle the increased incidence of iron deficiency anemia, especially in the western countries 11. Iron deficiency anemia is the most common micronutrient deficiency affecting mostly lower socioeconomic populations of developing countries 23. The non-nutritive toxic metals which are known to have deleterious effects even in small quantities (below 100 ppm) are As, Sb, Cd, F, Pb, Hg, and Se 25,26.
Therefore the manufacture of juices requires special attention in terms of purity and the sources of water and its purification are crucial for maintaining quality and safety and quality control and market watching will be do more monitoring for food especially fruit juice. The aim of present study is to compare the result values with acceptable values were proposed by food administration of American and WHO and the corresponding values of different countries available in the literature.

**Methods and Material**

Sixty-nine samples of commonly consumed fruit juices were analysis to determinate the concentrations of Iron Fe, Zinc Zn, Magnesium Mg, Sodium Na, Potassium K, Calcium Ca and heavy metal manganese Mn, mercury Hg, Lead Pb by using Atomic Absorption Spectrophotometer (AAS) agricultural college, main laboratory research / university of Salahadin. Digestion of Fruit Juices Samples performed at Halabja technical agricultural college in Halabjah city. All glassware’s washed with 1% nitric acid followed by demineralized water. 10 mL of concentration Sulphoric acid (H2SO4) was added to 2 mL of the fruit juice, then added 5 mL of hydrogen peroxide (H2O2), digestion of samples in a Kjeldahl digestion tube, the solution was heated on a Kjeldahl heater for 30 min or higher degree then turn into half and heated for 15 minutes. This was then allowed to cool in room temperature and transfer into a 100 mL volumetric flask and made up to mark with deionized water, which brought from the foundation of Kurdistan for strategic and research, then stored in a Pyrex glass bottle container transport into agriculture college, university of Erbil for analysis of Zn, Mn, Hg, Mg, Fe metal by atomic absorption spectroscopy model Alpha-4 and Na, Ca, K, flame photometer model Jenway PFP-7.

**Result and Discussion**

The results of the determination of the concentrations of metal in the fruit juice are shown in tables 1 , maximum, minimum, mean, and detected percentage of contents represent in table 2 .The mean value of Iron Fe, Zinc Zn, Manganese Mn, Magnesium Mg, Sodium Na, Potassium K, Calcium Ca and heavy metal mercury Hg, Lead Pb 0.421 , 0 ,0, 0.16 , 4.188 , 3.348 , 7.362 ,0.029 , 0.578mg/L) respectively. The ranges of the metals detected in all samples of fruit juice were; Iron Fe (0-2), Zinc Zn (0), Manganese Mn (0), magnesium Mg (0-0.6), Sodium Na (0-22.9), Potassium K (0-19.2), Calcium Ca (0-29) and heavy metal mercury Hg(0-0.25), Lead Pb (0-4) mg/L.

<table>
<thead>
<tr>
<th>Metals</th>
<th>Pb</th>
<th>Fe</th>
<th>Zn</th>
<th>Mn</th>
<th>Mg</th>
<th>Na</th>
<th>K</th>
<th>Ca</th>
<th>Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.578</td>
<td>0.421</td>
<td>0</td>
<td>0</td>
<td>0.160</td>
<td>4.188</td>
<td>3.348</td>
<td>7.362</td>
<td>0.029</td>
</tr>
<tr>
<td>Min</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Max</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0.6</td>
<td>22.9</td>
<td>19.2</td>
<td>29</td>
<td>0.25</td>
</tr>
<tr>
<td>Percentage % detected</td>
<td>30.43%</td>
<td>69.57%</td>
<td>0%</td>
<td>0%</td>
<td>84.06%</td>
<td>72.46%</td>
<td>72.46%</td>
<td>57.97%</td>
<td>21.74</td>
</tr>
</tbody>
</table>

**Mercury (Hg)**

Mercury was detectable in (21.74 %) of samples as shown in table 2 above and has concentration (0 – 0.25 ppm). The maximum value for Hg was found in sample FJ1, FJ13 and 21 was 0.25 ppm, the mean value detected 0.029 ppm. All detectable value was high compared to the maximum permissible limit for mercury is 0.01 ppm according to the national standard of China on Maximum Levels of Contaminants in Foods, a maximum level for mercury in fruits is 0.01 mg/kg (NSCMLCF, 2005). The average daily intake of mercury is reported to be between 0.002-0.02 mg (GMACE, 2001). Mercury is one of the most toxic elements among the studied heavy metals and exposure to high level of this element could permanently damage...
the brain, kidneys and developing fetus.

Sodium (Na)

It detected in 72.69% of samples and the mean of detected was 4.188 mg/dl, the range was (0-22.9 mg/dl).

Lead (Pb)

Lead was detectable in (30.43 %) of samples as shown in table 1 and 2 above and has concentration (0–4 ppm), The maximum value for pb was found in sample FJ7 was 4 ppm, the mean value detected 0.578 ppm. All detectable value was high compared to the maximum permissible limit for lead is 0.01 ppm set by WHO. The mean value was LESS than that reported in Accra Ghana fruit juice 1.59 mg/L.

Iron (Fe)

Iron was detectable in (69.57%) as show in table 1 above and has concentration (0 – 2 mg/L), The maximum value for Fe was found in sample FJ5, 6, 27, 31 was 2 mg/L, the mean value detected 1.34mg/L. The values of iron were lower compared to 0.020 –2.090 mg/L for non-canned and 0.020 – 2.460 mg/L for canned beverages 12, and lower than (3.13-5.48 mg/L) 3 but higher than values (0.11 – 0.28 mg/L reported) 14. All the fruit juice samples had iron concentration higher than the maximum limit permitted by WHO 23 except undetected samples. Iron is important in many biological processes because it is an ideal oxygen carrier and because it can function as a protein-bound redox element. Iron deficiency is common worldwide and in infants can cause severe neurological deficit 17. Minerals play an important role in maintaining proper function and good health in the human body.

Calcium (Ca)

The values of Calcium were higher than 2.763 – 13.143mg/L 5 while lower compared to 0.28 – 262 mg/L [14], Calcium is needed for the formation and maintenance of bones, the development of teeth and healthy gums. It is necessary for blood clotting, stabilizes many body functions and is thought to assist in preventing bowel cancer 2. It has a natural calming and tranquilizing effect and is necessary for maintaining a regular heartbeat and the transmission of nerve impulses. The required amount includes: 1,000 mg/day for people aged 19-50 years and 1,200 mg per day for people over the age of 51 years. The maximum level of calcium is 2.5 g/day 18.

Potassium (K)

The potassium detected in 72.46% of samples and the rang were (0-19.2 mg/dL). The values of potassium were lower compared to 2.0 to 110 mg/L 5. Potassium concentrations of the fruit juices were relatively low compared to the recommended daily intake (RDI) of potassium. The RDI of potassium ranged between 1600 - 5000 mg/day. Potassium is the major intracellular ion, intimately related to sodium movement out of the cell via Na/K ATPase.

Manganese (Mn)

Manganese was not detectable in any samples as shown in table 1 above,deficiency in manganese leads to various health problems, which may include bone malformation, eye and hearing problems, high cholesterol levels, hypertension, infertility, weakness, heart disorders, memory loss, muscle contraction, tremors, seizures. It could also result in decreased learning ability in school-aged children and increase the propensity for violence in adult.

Zinc (Zn)

Zn is one of the important trace elements that play a vital role in the physiological and metabolic process of many organisms. Nevertheless, higher concentrations of Zn can be toxic to the organism. Zinc was not detectable in any samples as shown in table 1 above also the Zn concentration below the limits imposed by USEPA (2008) and WHO. The maximum contaminant limit for zinc is 5.0 ppm . The result was less than that reported 0.301ppm 38 and reported in Accra Ghana fruit juice 3.33 ppm. Zinc is involved in numerous aspects of cellular metabolism. It is required for the catalytic activity of approximately 100 enzymes and it plays a role in immune function, protein synthesis, wound healing, DNA synthesis, and cell division.

Magnesium (Mg)

Manganese is known as trace elements. According to the USDA the daily recommended intake of manganese is 2.3 mg/day for adult males and 1.8 mg/day for female. The consumption of dietary trace-elements will help to prevent free radical damage. According to Olajire and Azeez 15, trace-elements have the ability to scavenge free radicals by inhibiting the initiation step or interrupting the propagation step of oxidation of lipid and as protective antioxidants which slow the rate of
oxidation by several actions.

**Conclusion**

Iron, calcium, zinc and potassium are essential elements needed for the general health. Although their values were low compared to the acceptable total intakes, their presence contributes to the daily iron, calcium, zinc and potassium sources needed in the body. The lead detected in 15 samples (44.11%) were above the MCL of 0.01 mg/L while manganese of fruit juice were not detected in any samples and MCL of manganese is 0.05 mg/L.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Sulaimania Polytechnic University (SPU), Kurdistan, Iraq and all experiments were carried out in accordance with approved guidelines.

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A Questionnaire-Based Survey Assessment of Iraqi Dentists Using Repair Versus Replacement of Defective Composite Restoration

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Abstract

Tooth restoration one of the most common procedures in dental practice. The replacement of the entire restoration leads to loss of tooth structure and increase risk of pulp injury; replacement is also time consuming and costly. According to the minimally invasive approach when minimal defects, repair is the better choice than the total replacement of the restoration.

This study aims to evaluate repair rating versus replacement treatment procedure for defective composite fillings among Iraqi dentists.

Material and methodology: A questionnaire survey were designed and distributed to 184 post-graduate dentists in Iraq. The inquiry pertained general information; including their clinical experience in years, their preference in terms of direct composite restoration, repair or replacement of old restorations, whether they taught the indications for repair, and the selection type of composite for repair.

Result of 200 questioner’s disrepute, 184 questionnaires were completed and collected. The data revealed that the respondents with 1-5, 6-15 and 16-25 years of experience were 62 (33.7%), 75 (40.8%), and 47(25.5%) of total respondents, respectively. The total 174 dentists (94.6%) prefer using tooth-colored restorations and 10 (5.4%) dislike this approach. Result clearly indicate that most of the dentists choose replacement for any fractured or discolored restorations. The data showed that 59.2% of dentist are not familiar with repair, and 40.8% dentists are familiar. The result revealed that 82.1% the dentists were not trained on repair during their undergraduate study. Generally, they had no ideal standard to replace or repair depending on their clinical experience. Therefore, clear criteria and guidelines for replacing and repairing should be developed and followed in post-graduate courses.

Key words: Composite restorations, repair or replacement, Iraqi dentists.

Introduction

The requirement for an ideal esthetic material of tooth restoration has resulted in considerable improvements in materials and techniques. Various resin-based composite (RBC) materials have been recently introduced, offering improved esthetic and physical material properties¹.

Direct RBC restorations, as well as other forms of restorations, generally suffer from deterioration due to wear and tear². The restoration of large defects in posterior teeth were tested to the maximum properties. The longevity of RBC materials may be compromised when using unprecise incremental technique³, resulting in higher failure rates⁴.

The clinical traditional management approach of restorations, in tooth exhibiting signs of defective margins (marginal deterioration, secondary caries or discoloration) is usually performed by total restoration replacement⁵. This approach leads to the significant disturbance of healthy tooth structure when the preparation area is enlarged, resulting in negative effect on tooth longevity. Moreover, the possibility of pulp fatal injury is increased ². Approximately 56% of restorations reportedly were refilled rather than restorations for new
lesions of caries\(^6\).

Most defects in restorations, other than those caused by sudden impact fracture, gradually develop with time\(^{(7,8)}\). This providing an opportunity to classify the problems and then undertake minimal intervention to correct the defects.\(^2\) Caries occurring at the margins of a restoration is considered a new lesion rather than recurrent caries. Therefore, localized repair rather than total restoration replacement is needed.\(^{(2,3,8)}\)

When a minimal or localized defect appeared at one restoration region, repair is better option than total replacement of the restoration\(^9\). Repair usually involves partial replacement of a restoration using the same or different restorative materials. Practically repairing consume short operating time, so it can be used for uncooperative patients or those suffering complex medical histories. Furthermore, the repair may be performed without local anaesthesia and complex operative procedures\(^{10}\). RBC restoration repairing may be reduced to refurbishment only. Usually refurbishment involve the refinishing or resurfacing of a restoration with or without recountouring. Refurbishment usually involves refinishing or resurfacing with or without recountouring. Refinishing can be performed only to the margins of the restoration, while resurfacing may involve part or all of the exposed surfaces of restoration\(^3\). Although numerus surface treatment modalities have been introduced, the interface between the aged and repair composite material is considered weak link. However, none of these modalities can been regarded as the golden standard\(^{(11,12)}\).

Owing to the unavailability of practical guidelines, so the decision between replacing or repairing usually depends on judgment of the clinician, according to their clinical experience.\(^{10}\)

The present study aims to survey the preference of Iraqi dentists between repair or replacement in dental restorations.

### Material and Method

A survey questionnaire consisting of eight questions was designed and distributed to 200 post-graduated dentists engaged the public dental service. The respondents were informed that their demographics will be kept confidential. The survey inquired a general information of the participants, including years of clinical experience, their preference among direct composite restoration, repair or replacement of old restorations, and their preference of special composite material for repair. The collected questionnaires results were analyzed. The following percentage results were obtained: (1) Years of experience, (2) Prefer tooth colored restoration, (3) Preferred restoration methods if fractured, (4) Preferred restoration methods if discolored, (5) Familiarity with repair, (6) Trained on repair during under graduate study, (7) Repair decision influence by fillings age, and (8) Selection of special composite.

### Result

The complete questionnaire received from different dental health centers were one hundred eighty-four (N = 184) out of two hundred responded. The data of 184 revealed that dentists had experience of 1-5 years were 62 (33.7%), 6-15 years were 75 (40.8%), 16-25 years were 47 (25.5%). 174 dentists (94.6%) prefer using tooth-colored restorations and 10 (5.4%) dislike table\(^{(1)}\). This table also indicated, that when there is any fracture in the restorations, 66.1% of dentists choosed restoration replacement. 20.2% of dentists their decision varies between replacement or repair according to the case, but 13.7% prefer repairing the defected filling. The data showed 59.2% of dentist not familiar with repair, and 40.8% dentists were familiar. the questioner revealed that 82.1% the dentists were not trained on repair during their undergraduate study while only 17.9% were trained, figure (1).

![Figure (1): Characteristics and responses of selected sample of dentists.](image-url)
The results demonstrated that when there was restoration discoloration, 53.3% of the participants whom familiar with repair preferred replacement over repair and 18.7% preferred repair of the restoration. The trained undergraduate dentists, 48.5% from them prefer replace over repair, while 24.2% their decision was according to the case, table (1)

**Table 1: Distribution of participants according to preference of restoration method for discolored filling and to studied variables:**

<table>
<thead>
<tr>
<th>Preference of restoration method</th>
<th>Repair</th>
<th>Replace</th>
<th>According to case</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables</td>
<td>N=30%</td>
<td>N=115%</td>
<td>N=39%</td>
</tr>
<tr>
<td>Familiarity to repair</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>14</td>
<td>40</td>
<td>21</td>
</tr>
<tr>
<td>No</td>
<td>16</td>
<td>75</td>
<td>18</td>
</tr>
<tr>
<td>Trained on repair during undergraduate study</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8</td>
<td>16</td>
<td>9</td>
</tr>
<tr>
<td>No</td>
<td>22</td>
<td>99</td>
<td>30</td>
</tr>
</tbody>
</table>

While if there is fractured filling there were 78.9% of dentist which there not familiar to repair prefer replacement over repair of restoration 37.3%of dentists familiar with repair preferred replacement of filling. Table (2)

**Table 2: Distribution of participants according to preference of restoration method for fractured filling and to studied variables:**

<table>
<thead>
<tr>
<th>Preference of restoration method</th>
<th>Repair</th>
<th>Replace</th>
<th>According to case</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables</td>
<td>N=24</td>
<td>N=121</td>
<td>N=37</td>
</tr>
<tr>
<td>Familiarity to repair</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>13</td>
<td>35</td>
<td>26</td>
</tr>
<tr>
<td>No</td>
<td>12</td>
<td>86</td>
<td>11</td>
</tr>
<tr>
<td>Trained on repair during undergraduate study</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>5</td>
<td>21</td>
<td>6</td>
</tr>
<tr>
<td>No</td>
<td>20</td>
<td>100</td>
<td>31</td>
</tr>
</tbody>
</table>

In our study we shown that if repair decision influenced by fillings age some participate say yes and others no about the dentists which there show that there are a relation between filling age and repair decision 54.7% of them are not familiar with repair and 45.3%not familiar with repair and 81.3%are not trained on repair during under graduate and 18.8%are trained on under graduate study table (3)
Table 3: Distribution of participants according to influence of filling’s age upon repair decision and to different factors:

<table>
<thead>
<tr>
<th>Variables</th>
<th>Repair Decision influenced by filling’s age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Experience (y)</td>
<td></td>
</tr>
<tr>
<td>• 1-5 y</td>
<td>44</td>
</tr>
<tr>
<td>• 6-15 y</td>
<td>51</td>
</tr>
<tr>
<td>• ≥16 y</td>
<td>33</td>
</tr>
<tr>
<td>Familiarity to repair</td>
<td></td>
</tr>
<tr>
<td>• Yes</td>
<td>58</td>
</tr>
<tr>
<td>• No</td>
<td>70</td>
</tr>
<tr>
<td>Trained on repair during under graduate study</td>
<td></td>
</tr>
<tr>
<td>• Yes</td>
<td>24</td>
</tr>
<tr>
<td>• No</td>
<td>104</td>
</tr>
</tbody>
</table>

Discussion

The results revealed that 94.6% of the participants prefer tooth-colored restoration as requirement for aesthetic dental restorations, and the RBC was the restorative material of choice for both anterior and posterior teeth. These results were consistent with those of Valeria V. Gordan Cynthia W. Garvan, Paul K. Blaser, Eduardo Mondragon; Ivar A. Direct composite restorations (tooth-colored, with minimum tooth preparation) exhibited good performance. Thus, these materials are currently used for various purposes. Modern bonding materials and techniques decry polymerization shrinkage, microleakage and the occurrence of secondary caries. Therefore, composite resins are preferred by most practitioners for anterior and posterior restorations.

Given the lack of acknowledgement among dentists about the advantages of repairing resin composite restorations, some dental practitioners are possibly unaware of the repair option. However, others consider the contraindications to repair: patient disinclination to accept a repair, irregular attendance, high risk of caries, presence of caries that undermine restoration, and history of previous repair failure. In this study many participate considered the choice of repair or replacement depending on the restoration case. Therefore, a large percentage of dentists prefer replacement over repair. This finding consistent with the study of Tays et al. who considered that repair is not the standard of care and patchwork dentistry.

The result of this research generally disagreed with those of other workers, which indicated an agreement among academicians in conservative dentistry worldwide in repairing rather than directly replacing resin composite restorations. By contrast, this study revealed that the majority of dentists did not receive didactic information on repair during their undergraduate study. Only 17.9% of dentists claimed that they were taught and trained in composite repair during the BDS study. While 40.8% performed repairs in their practice.

Regarding discoloration of restoration, most dentists prefer replacement. Discoloration may be due to incorrect shade selection in the placement of a composite restoration, which may be managed by resurfacing using a different shade of composite material. If possible, the same restorative material should be used as the composite substrate. However, this condition might be impossible if the restoration was performed by a different practitioner, materials used were not recorded in the patient’s notes, or the previous material is no longer commercially available.

In this research, a high percentage of dentists prefer the replacement of fractured tooth-colored restoration. This preference may be attributed to fracture of tooth.
tissue adjacent to a restoration, which may be occurred due to parafunctional activity or trauma, the subsequent to the damaging effects of polymerization stresses in resin composites at the time of restoration placement. A repair may be indicated when the fracture accurately diagnosed, and the risk for further fracture minimized. This investigation underlines the need for clinical studies on repairs and the requirement for general guidelines of the repair indications and technique options.

Conclusions

The choice between repairing or replacing a defective RBC restoration is multifactorial, and the answer of many participants was based on each case treatment. This study highlighted lack of acknowledgment among dentists about the advantages of repairing RBC. Evidently, most of the participants were unaware of repair indications and techniques similar to those of other countries in the world.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Baghdad university college of dentistry and all experiments were carried out in accordance with approved guidelines.

References

Assessment of the Level of Spirituality among the Patients with Major Depressive Disorder in Sulaimani City

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2 College of Nursing/University of Sulaimani

Abstract

Background: Major depressive disorder has been associated with greater morbidity and mortality. Many researchers have been sought complex association between depressions with spirituality. The levels of severity of such factor can play role in the causes, recovery and predictor of depression.

Objectives: The main objective of this study is to assess the levels of spirituality.

Method: A quantitative descriptive design, conducted at psychiatric clinic in Ali Kamal medical consultation center in Sulaimani City. A non-probability, convenient sampling was recruited of 150 patients with major depressive disorder attending the psychiatric clinic. The data were collected from December, 21st, 2017 to April 1st, 2018 by the researcher of current study through the utilization of structured Face-to-Face interview guided by the questionnaire.

Result: The result shows that the patients with major depressive disorder experience high spirituality value.

Conclusions: The study conclude that high spirituality value on its own the strongest significant factor may represents emotional source and possibly used to handle or cope with depressed mood by the patients.

Keywords: Spirituality, Depression, Patients, Major depressive disorder

Introduction

The World Health Organization, 1946 defined health as state of complete physical, mental and social well-being and not merely the absence of disease or infirmity. However, more recent definitions of health have included emotional and spiritual dimensions. To ignore any of these components would omit significant part of well-being, with equally significant consequences.

Spirtuality is broad concept comprise many perspective in general, it includes a sense of connection to something bigger than ourselves, and it typically involves a search for meaning in life. As such, it is a universal human experience, something that touches us all. Individuals may describe a spirituality experience as sacred or transcendent or simply a deep of aliveness and interconnectedness.

Depression has been studied in relation to spirituality and it appears that spirituality does help people maintain their mental health. Spirituality may play a role in depression prevention and recovery. Some studies indicate that more spirituality involved individuals experience fewer depressive symptoms and faster recovery from a depressive disorder than those with less spirituality involvement. It has been suggested spirituality acts as a coping resource or protective factor against depression.

According to Russell’s model as well-being spirituality is not necessarily religious aspect, but rather an individual’s philosophy; value and meaning of life. It is defined as a personal connectedness with a higher divine nature. Such connectedness may contributes to lessening depression.

18 have assessed the value of the spirituality teaching of 8- week program for adult diagnosed with major depression of mild to moderate, results showed...
significant decreased in severity change, response and remission. Also, reported that spirituality is a significant predictor of depression should be considered on area of study.

The previous studies sought to study depression in terms of spirituality is limited. Furthermore no study has investigated the levels of spirituality in outpatients with major depressive disorder in the psychiatric-mental health nursing in Sulaimani city and even in Iraq.

Today psychiatrists, psychologists and clinicians are increasingly respond to the idea that spirit as integral in the life of the person and believe that spirituality are important in the life of their patients. The American Counseling Association- defining the spirituality as a capacity and tendency that is innate and unique to all people. This spiritual tendency moves the individual toward knowledge, love, meaning, peace, hope, transcendence, connectedness, compassion, wellness, growth and development of a value system. Spirituality encompasses a variety of phenomena, including experiences, beliefs, and practices. Spirituality is approached from a variety of perspectives, including psycho-spiritual, religious and transpersonal, while spirituality is usually expressed through culture.

Individual’s spiritual-health have two characteristics; internal and external. Internally, spiritual health seems to provide the individual with life purpose, ultimate meaning oneness with nature and beauty, a sense of connectedness with other, deep concern for a commitment, peace, hope and fulfillment. Externally, spiritual-health of individuals expresses themselves through trust, honesty, integrity, altruism, compassion and service. In addition, these characteristics regularly communicate with higher power or larger reality that transcends an observable physical reality.

The role of the spirituality in life not optional, it is a response to the need for human wholeness. To not miss an essential part of the person, in the treatment of illness, it is important to attend to the spiritual dimension of a person.

Spirituality and religiosity are related and overall but is not the same. Religiosity is a specific set of beliefs and practices usually within an organized and formal group or it may be a less formal and more individual set of beliefs and practices.

Religion is institutionalized spirituality, thus there are different sets of beliefs, traditions, and doctrines. Spirituality is the common factor in all these religions.

In general, Islamic theory holds that each person emprises four parts; the heart, the intellect, the spirit (Ruh) and the self (Nafs). The spirit contains a template of potential for the person. And the self encompasses the biological and psychic aspects of the individual (our animal nature). The four parts of the person are in harmony through belief, worship and submission to Allah.

Although hospital for psychiatric care appeared in Islamic countries early in the 8th century, Muslims generally may see mental health difficulties as due to spiritual problems or weakness in faith. Islamic counselors working with Islamic clients generally see their spirituality; religion is as a resource that helps guard against problem like depression and suicide, as well as providing coping resources for other types of problem.

view of the spiritual well-being concept that, in addition to desires for pleasure or self-fulfillment, humans have needs for transcendence, “the sense of well-being that we experience when we find purposes to commit ourselves to which involve ultimate meaning for life”. This type of well-being provides integration, harmony, and freedom within the personality and it involves two types; religion well-being which is a vertical relation or well-being in connection to God, and the second type is existential well-being which includes a horizontal relation to the world including a sense of life purpose and satisfaction.

In this regard stated that some major spiritual issue include the fear of death and loss, both of self and others. Spirituality allows one to cope with these feelings by providing a sense of hope and meaning to experience that would otherwise be crippling. Having a spiritual understanding that one’s connection with creation is more than merely physical helps to ease the fear and pain of loss, feeling connected to the divine eases feelings of abandonment, grief, and alienation, as well as promoting a sense of self-acceptance. Spirituality is thought to be a key component in the healing process and an integral part of the client treatments plan.

Spiritual / religious coping is widespread. In a study published by found that 90 percent of Americans
turned to their spiritual belief to cope with stress. This is also true in clinical settings. A study of 330 hospitalized medical patients found that 90 percent reported they used spiritual and religion practices to cope at least a moderate extent, and over 40 percent indicated that their spiritual belief were the most important factor that kept them going 17. Those with mental problems often rely on spirituality resources. 23 found that 80% of patients (406) with persistent mental illness relied on spiritual beliefs to cope. In Australia, 11 found that 79% among 79 psychiatric patients thought their therapist should be aware of their spiritual beliefs and 67% percent indicated that spirituality helped them to cope with psychological pain.

16 examined the relationship between spirituality and mental health, focusing on depression, and reported statistically significant positive associations between spirituality involvement and better health. Spirituality in mental health plays an important role. For many afflicted individuals, their spirituality gives them a powerful sense of hope in the face of an often devastating and chronic illness 7.

Method

A quantitative-descriptive design was used in order to achieve the aim of the study by using assessment technique to describe the studied variables which includes spirituality on community Patient with major depressive disorder. The study was carried out from December, 10th, 2017 to October, 1st, 2018. This study carried out at Psychiatric clinic in Ali Kamal medical Consultation Center which is affiliated to the Teaching Hospital in Sulaimani City in Kurdistan Region of Iraq.

A non-probability, convenient sampling of 150 patients previously diagnosed with major depressive disorder were recruited from consecutively attended the psychiatric department during the period from December 27th, 2017 to March, 21st, 2018.

A questionnaire was developed to measure the variables underlying the present study mainly to assess spirituality among patients diagnosed with major depressive disorder. Spiritual well-being scale (SWBS) (Elison-paluzian, 1982) used in this study. The validity of the questionnaire determined through a panel of 20 experts. Statistical package for social science (SPSS) version 22 is used for data analysis.

Result

Table 1 the significant difference between calculated mean and theoretical mean in spirituality measures for the study sample, using -test

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sample</th>
<th>Calculated mean</th>
<th>standard deviation</th>
<th>theoretical mean</th>
<th>value</th>
<th>Level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spirituality</td>
<td>150</td>
<td>72.626</td>
<td>11.398</td>
<td>60</td>
<td>13.567</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table (1) shows statistically significant difference was found between calculated mean ($= 72.626$) and theoretical mean ($x=60$) ($-test = 13.567, ± 11.398$) at <0.001 level in spirituality measure. The results in this table indicate that the difference was in favour of calculated mean which means that the level of spirituality, in general is high among study sample for patients with major depressive disorder.

Table 2 Distribution of sample according to the levels of spirituality

<table>
<thead>
<tr>
<th>Statistical Indicator</th>
<th>Low level (20-40)</th>
<th>Medium level (41-69)</th>
<th>High level (70-100)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>3</td>
<td>45</td>
<td>102</td>
<td>150</td>
</tr>
<tr>
<td>Percentage</td>
<td>%2</td>
<td>%30</td>
<td>%68</td>
<td>%100</td>
</tr>
</tbody>
</table>
Table (2) appears the distribution of the patients according spirituality-well-being scale index. The table reveals that highest percentage (68%) of the total patients were high level of spiritual well-being and the mean score ranged from (70-100), and the lowest percentage (2%) of the total patients were low level of spiritual well-being the mean score ranged from (20-40).

Table (3) The significant difference of spirituality in regard to patient’s gender factor among sample

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group</th>
<th>Number</th>
<th>Mean score</th>
<th>Standard Deviation</th>
<th>value</th>
<th>Level of significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spirituality</td>
<td>Males</td>
<td>99</td>
<td>73.64</td>
<td>8.81</td>
<td>1.534</td>
<td>Nonsignificant</td>
</tr>
<tr>
<td>Spirituality</td>
<td>Females</td>
<td>51</td>
<td>70.64</td>
<td>15.12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The table (3) appears that there was statistically non-significant difference between mean score of spirituality in compare to patient’s gender because p-value was greater than common alpha 0.05 (- test = 1.534). The table reveals that gender factor is not impact factor affecting the spirituality in major depressive disorder among study patients.

Table 5 the significance differences of mean score of spirituality in regard to suicide attempt factor among patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Suicide attempt</th>
<th>Number</th>
<th>Mean score</th>
<th>Standard Deviation</th>
<th>value</th>
<th>Level of significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spirituality</td>
<td>No</td>
<td>102</td>
<td>73.83</td>
<td>12.11</td>
<td>1.907</td>
<td>Nonsignificant</td>
</tr>
<tr>
<td>Spirituality</td>
<td>Yes</td>
<td>48</td>
<td>70.06</td>
<td>9.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spirituality</td>
<td>Yes</td>
<td>48</td>
<td>12.93</td>
<td>4.60</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (5) shows that there was statistically non-significant difference mean score of spirituality in compare to patients non suicidal and suicidal attempt, because p-value was greater than the common alpha 0.05 (=1.907, at p>0.05). Table 5 reveals that the suicidal attempt is not affecting spirituality, which reveals that spirituality, is self-coping against suicide attempts.

Discussion

The results of the present study showed that the observed (calculated) mean score (72.626) of spirituality was significantly (p<0.001) higher than the theoretical mean score (60) 11.398 and the majority of the study depressed patients (68%) have high spiritual level in comparison to the standards scoring (70-100) of spirituality scale and only few of them (2%) with low level of spirituality. The frequent finding of high spirituality level among depressed patients in the current study was similar to the findings from another study carried out by 15 study in Iran and 4 study in USA. They found that spirituality level was significantly high among patients with major depressive disorder.

The possible explanation of the result of this study that the collaborative relationship that depressed patients have with God may counteract the culture belief of fatalism 4. The findings of this study therefore add evidence that high spirituality level common state experience by the study patient, with major depressive disorder living in the community.

It showed that gender and suicide attempts are non-significant factors affecting spirituality levels. Similarly, 4 showed that gender was non-significant impact factors affecting the differences in spirituality score levels. In contrast to the results of this study 15 identified that the gender as a factor effecting spirituality levels.
Suicidal attempt also is non-significant factors effecting the differences in spirituality levels (p>0.05). found that no support for spiritual mobilization hypothesis during illness or that spirituality/ religious served as protective factor, and also found that distress increases over time and proposed that the strength of religion/ spirituality beliefs prior to the illness and the mobilization of these beliefs in absence of illness a strong base during the crises promoted distress Instead.

**Conclusion**

Most of patients were male, unemployed, living in urban areas, practicing religious activities and their mean of age 37.5 years. They were less admitting to hospital although the long duration of illness ranged from 1-25 years. The patients experience high level spirituality value. The level of spirituality value affected by age, marital status, level of education, residential area, religious status and duration of illness.

Patient religious status is significant factors effecting the variation of studied variable spirituality, but patient suicide attempt and number of attempts are not associated with the spirituality value.

High spirituality value on its own the strongest significant factors may represent emotional source and possibly used to handle or self-cope with depressed mood by the patients.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of Nursing/University of Sulaimani, Iraq and all experiments were carried out in accordance with approved guidelines.

**References**


Isolation and Diagnosis of Some Dermatophytes Using Nitrogen Base Tracking Technique (Sequence)

Saba Kadhim Naser1, Meethaq Staar Abood2

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Abstract

A total of, 162 Samples of those infects with dermatophytes at Imam Hussein Teaching Hospital in Nasiriyah city for the purpose of diagnosing some samples in a sequential manner , The samples included skin skimmers, nail clippers, hair pieces, Isolation and diagnosis of samples was carried out by conventional methods and then confirmed the diagnosis in a way that follows the nitrogen bases, where 10 samples were diagnosed and recorded in the gene bank , Trichophyton was the more frequent , than Epidermophyton,A number of Candidia yeasts were also diagnosed in the traditional methods of appearance , It was a ratio of T. rubrum (28%) than T. interdigitale (24%), than T. mentagrophytes (21%), and also E. floccosum in the rate of (25%) , There are also ratios for the emergence of types of yeasts as a proportion C. albicans (32%) , Than C. parapsilosis (25%) , Than C. kruzi (22%) , and also C. dubliirensis in the rate of (19%) , Thes samples were collected from different ages and from both sexes .

Key words : Dermatophytes, Nitrogen base tracking technique (Sequence).

Introduction

Dermatophytes are a group of fungi that have the ability to attack keratinized tissues of humans and animals such as skin, hair and nails, causing dermatophytes, Thes fungi comprise three species (Trichophyton Epidermophyton, Microsporu) .

Trichophyton is characterized by the formation of large conidates and small conidaes with a smooth wall and this genus includes a number of species that have the ability to infect the skin, hair and nails .

Skin infections are a common infection in humans for a long time as millions of people in the world are exposed , It causes skin infections known as ringworm(Tinea) or ringwor infection (Tinea) or skin mycosis in humans and animals , It has the advantages of being a keratin-loving keratin (Keratinophilic) and his analyzer (Keratinolytic), but has no ability to penetrate deep tissues below the keratinized layer as most of them are unable to live at high temperature such as body temperature .

The incidence rates vary depending on the location of the infection in the body and age, as the incidence of scalp (Tinea capitis) in younger ages is more than in adults , Asat and his group in 1996 and the researcher (Chin,2000) reported that the relative resistance to infection is due to the long chains of saturated fatty acids found in sebum produced after puberty , ringworm (Tinea corporis) occurs in warm areas of the world and affects more children, especially those in direct contact with animals and adults, especially in those with excessive sweating . While ringworm (Tinea pedis) affects athletes in general because of its spread in swimming pools and clothing strikes, and crowded and public place (such as schools, hospitals, and public parks) are preferred sites for the emergence and wide variety of pathogenic and opportunistic fungi .

There are many laboratory methods used to diagnose skin fungi one of these methods is forming on food circles , There are also special circles used to diagnose skin fungi called Dermatophytes Identification Media (DIM) in these settings, the diagnosis depends on the color variation of the fungal colonies , Diagnosis can also be made based on specific nutritional requirements for the growth of fungi, such as the need for the thymine compound T. violaceum , Currently, the PCR technique is used to diagnose fungi if the fungal colony lacks the diagnostic phenotypic characteristic or
the newly developed fungal colony or if the fungus is dead. 

Materials and Method

Collect samples

About 162 samples (hair pieces, demabrasion, and nails) were collected from patients attending Imam Hussein Teaching Hospital in the Dermatology Consultation Department in Thi-Qar Governorate. (Gender, age, area of residence, injury area as well as the work of the injured person and date of sample collection).

Examination of samples

Direct microscopy of samples The samples were transferred to the college laboratory as they were taken from the affected skin, hair and nails and added drops of potassium hydroxide solution at a concentration of 10% after being placed on a glass slide and left for 5 minutes of time. In microscopic examination using a complex optical microscope and at a power of (40X).

Cultivation of specimens

The remaining part of the samples is planted on the medium of Sabouraud Dextrose Agar with Cycloheximide and Chloramphenicol. The medium of Sabouraud Dextrose Agar added whit Cycloheximide and Chloramphenicol to prevent the growth of opportunistic bacteria and fungi. Prepare the medium by melting 65 g of sabouraud dextrose agar in 1000 ml distilled water, sterilize with anodized and cool to 45°C and add 0.05 of chloramphenicol antibiotic to inhibit bacterial growth and 5.0 cycloheximide to inhibit the growth of unsatisfactory thrush, use this medium to isolate grow and preserve fungal isolates.

Diagnosis

Cultivated dishes were examined five days after transplantation and then left for three weke at 28°C to allow more fungi to grow and appear, the fungi were then isolated from dishes containing SDA medium, the isolates are then purified and transferred from the colonial parts to the slanted media, it was incubated at 25°C and then kept in the refrigerator at 4°C to 6°C until later use. All fungi were laboratory diagnosed based on the cultivative and phenotypic qualities and with the help.

DNA sequencing method

DNA sequencing method was performed for species typing of positive Trichophyton sp. isolates as following step:

1- The PCR product of 18S ribosomal RNA genes were sent to Macrogen Company in Korea in ice bag by DHL for performed the DNA sequencing by AB DNA sequencing system.

2- The DNA sequencing analysis (Phylogenetic tree analysis) was conducted by using Molecular Evolutionary Genetics Analysis version 6.0. (Mega 6.0) and Multiple sequence alignment analysis based ClustalW alignment analysis and The evolutionary distances were computed using the Maximum Composite Likelihood method by phylogenetic tree UPGMA method.

3- The Trichophyton species typing analysis was done by phylogenetic tree analysis between local Trichophyton sp. isolates and NCBI-Blast known Trichophyton species.

4- Finally identified Trichophyton species isolates were submitted into of NCBI-GenBank to get Genbank accession number.

Medium Chrome Agar Candida

Uses to distinguish between the types of Candida, prepare the medium according to the instructions of the manufacturer by adding the powder medium (47 g) slowly to sterile distilled water Balmsdah (1000 ml) with stirring to homogenize the mixture and then heat to 100°C until large bubbles appear then cool to a degree 50°C water bath and poured into Petri dishes, then samples were planned on the surface of the medium and incubated at 37°C and you monitor growth for (24-48) hours and then observed the change in the colors of the colonines growing on this medium.

Results and Discussion

The results of laboratory transplantation on the medium of Sabouraud Dextrose Agar showed the presence of skin fungi in 70 samples of the total samples as shown in Table (1), While the number of samples in which Candida appeared was 40 samples as shown in Table (2), as for the results of the laboratory examination, 30 samples showed dermatophytes, Candida samples were 22, as shown in Table (3).
results of this table indicate that direct microscopic examination is highly sensitive in the diagnosis of skin injuries compared with implantation on the media\(^{19}\). They pointed out that the direct examination as a detection of the presence or absence of fungi, The emergence of negative results, whether in direct microscopy or laboratory transplantation for many reasons, the most important inaccurate diagnosis as the infection is not fungal, but are symptoms of allergic diseases, and also some patients resort to the use of treatments without consulting a doctor, which leads to influence the activity of the fungus causing the infection\(^{20}\), and not grow until transplantation\(^{21}\).

Table (1) number of fungal isolates isolated from skin, hair and nails

<table>
<thead>
<tr>
<th>Mushroom type</th>
<th>The number</th>
<th>Mean%</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. mentagrophytes</td>
<td>15</td>
<td>21</td>
</tr>
<tr>
<td>T. interdigitale</td>
<td>17</td>
<td>24</td>
</tr>
<tr>
<td>T. rubrum</td>
<td>20</td>
<td>28</td>
</tr>
<tr>
<td>E. floccosum</td>
<td>18</td>
<td>25</td>
</tr>
</tbody>
</table>

The total number of positive isolated fungi (70) was calculated by the following equation:

\[
\text{Number ÷ Total number × 100)}
\]

Table (2) number of candida isolates

<table>
<thead>
<tr>
<th>Candida type</th>
<th>The number</th>
<th>%Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>20</td>
<td>32</td>
</tr>
<tr>
<td>C. dubliensis</td>
<td>12</td>
<td>19</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>16</td>
<td>25</td>
</tr>
<tr>
<td>C. kruzi</td>
<td>14</td>
<td>22</td>
</tr>
</tbody>
</table>

The study showed the diagnosis of three species of the genus Tricchophyton from the samples included in the study which T. mentagrophyte and T. rubrum and T. interdigitale with one species of the genus Epiderophyton which is E. floccosum on the medium of Sabouraud Dextrose Agar added with Cycloheximide and Chloramphenicol, the study also showed the emergence of types of genus Candida, namely C. albicans and C. dubliensis and C. parapsilosis with C. kruzi it was grown on the medium Chrom Agar as shown in the following figures:

Molecular diagnosis using polymerase chain reaction

Nucleic acid was extracted from the isolates under study and the result was the emergence of DNA bundles clearly when imaging with UV light and for all the isolates selected using electrophoresis technique on acarose gel as shown in Figure (1).

The total number of isolated Candida was (62) and the percentage was calculated by the following equation:

\[
\text{Number ÷ Total number × 100)}
\]

Table (3) Results of microscopy and transplantation of samples

<table>
<thead>
<tr>
<th>Type of examination</th>
<th>Dermatophytes</th>
<th>Percentage</th>
<th>Candida</th>
<th>Mean%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct microscopy (KOH)</td>
<td>30</td>
<td>30%</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Fungal transplants results</td>
<td>70</td>
<td>70%</td>
<td>40</td>
<td>40</td>
</tr>
</tbody>
</table>

Figure (1) represents the shape of DNA produced using PCR technology
The results of the sequence for a set of studied samples

Ten specimens in the present study showed the new species of the genus Trichophyto sp. that were registered at the International NCBI- Genbank, the results revealed that there were many variables in many of the samples examined compared to the DNA sequence recorded in the bank.

![Multiple sequence alignment analysis of 18S rRNA gene in local Trichophyton sp. isolates and NCBI-Genbank Trichophyton sp. Isolates.](image)

Figure (2): Multiple sequence alignment analysis of 18S rRNA gene in local Trichophyton sp. isolates and NCBI-Genbank Trichophyton sp. Isolates. The multiple alignment analysis was constructed using ClustalW alignment tool in (MEGA 6.0 version). That show the nucleotide alignment similarity as (*) with substitution mutations in 18S rRNA gene.

**Trichophyton mentagrophytes isolate IQ-E5 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence**

GenBank: MN165773.1

FASTA Graphics

Go to:

LOCUS MN165773 324 bp DNA linear PLN 16-JUL-2019

DEFINITION Trichophyton mentagrophytes isolate IQ-E5 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence.

ACCESSION MN165773

VERSION MN165773.1

KEYWORDS .

SOURCE Trichophyton mentagrophytes

ORGANISM Trichophyton mentagrophytes

Eukaryota; Fungi; Dikarya; Ascomycota; Pezizomycotina;

Eurotiomycetes; Eurotiomycetidae; Onygenales; Arthrodermataceae;
Trichophyton.

REFERENCE 1 (bases 1 to 324)
AUTHORS Abood, M.S. and Naser, S.K.
TITLE Study on some genes associated with azole antifungal resistance of Dermatophytes
JOURNAL Unpublished

REFERENCE 2 (bases 1 to 324)
AUTHORS Abood, M.S. and Naser, S.K.
TITLE Direct Submission
JOURNAL Submitted (09-JUL-2019) Biology, College of Education for Pure Science/University Of Thi-Qar, Nasiriyah, Nasiriyah, Thi-Qar 00964, Iraq

COMMENT ##Assembly-Data-START##

Sequencing Technology :: Sanger dideoxy sequencing

##Assembly-Data-END##

FEATURES Location/Qualifiers
source 1..324
/orgianism="Trichophyton mentagrophytes"
/mol_type="genomic DNA"
/isolate="IQ-E5"
/isolation_source="skin lesion"
/host="Homo sapiens"
/db_xref="taxon:523103"
/country="Iraq"
misc_RNA <1..>324
/note="contains internal transcribed spacer 1, 5.8S ribosomal RNA, and internal transcribed spacer 2"

ORIGIN
1 tttcagagat gtgtctgtcg gagatttagt agtgaaaatc agttaaaaact ttgacaacg
61 gaattctggg ttccggcatc gatgaagaac gcagcgaaat gcgataagta atgtgaattg
121 cagaattccg tgaatcatcg aatctttgaa cgcacattgc gccccctggc attccggggg
181 gcgatactga aggtatgcatg cagatgttga aacagatgatg cagatatc tggtagacatg
ggcagttggg ggcagttggg ggcagttggg ggcagttggg ggcagttggg
ggcagttggg ggcagttggg ggcagttggg ggcagttggg ggcagttggg
ggcagttggg ggcagttggg ggcagttggg ggcagttggg ggcagttggg
241 ccgccccccc cggccccccc cggccccccc cggccccccc cggccccccc
cggccccccc cggccccccc cggccccccc cggccccccc cggccccccc
cggccccccc cggccccccc cggccccccc cggccccccc cggccccccc
301 tcagcttcc tagggaag agca

This fungus registered Trichophyton mentagrophytes as a local isolation in the gene bank with accession number MN165773

E05
Figure (3): Treatment of the sequence of nucleotides of the isolated local Trichophyton mentagrophytes
Table (4): the NCBI-BLAST Homology Sequence identity (%) between local Trichophyton sp isolates and NCBI-BLAST submitted Trichophyton sp. isolates:

<table>
<thead>
<tr>
<th>Trichophyton sp. Isolate No.1</th>
<th>Genbank Accession number</th>
<th>Genbank Accession number</th>
<th>Trichophyton sp. Isolate No.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichophyton mentagrophytes</td>
<td>MK312950.1</td>
<td>MN165768</td>
<td>Trichophyton sp. A05 isolate</td>
</tr>
<tr>
<td>Trichophyton mentagrophytes</td>
<td>MH791431.1</td>
<td>MN165769</td>
<td>Trichophyton sp. A06 isolate</td>
</tr>
<tr>
<td>Trichophyton mentagrophytes</td>
<td>MH791431.1</td>
<td>MN165770</td>
<td>Trichophyton sp. B06 isolate</td>
</tr>
<tr>
<td>Trichophyton mentagrophytes</td>
<td>KX906476.1</td>
<td>MN165771</td>
<td>Trichophyton sp. D05 isolate</td>
</tr>
<tr>
<td>Trichophyton mentagrophytes</td>
<td>MH791431.1</td>
<td>MN165777</td>
<td>Trichophyton sp. D06 isolate</td>
</tr>
<tr>
<td>Trichophyton mentagrophytes</td>
<td>MH791431.1</td>
<td>MN165773</td>
<td>Trichophyton sp. E05 isolate</td>
</tr>
<tr>
<td>Trichophyton mentagrophytes</td>
<td>KX906476.1</td>
<td>MN165774</td>
<td>Trichophyton sp. F05 isolate</td>
</tr>
<tr>
<td>Trichophyton mentagrophytes</td>
<td>KX906476.1</td>
<td>MN165775</td>
<td>Trichophyton sp. F06 isolate</td>
</tr>
<tr>
<td>Trichophyton rubrum</td>
<td>MF800872.1</td>
<td>MN165776</td>
<td>Trichophyton sp. G05 isolate</td>
</tr>
</tbody>
</table>

The reliance on molecular analysis of DNA sequences in the present study to diagnose dermatophytes is of great importance in supporting phenotypic diagnostic methods.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the University of Thi-Qar- College of Education for Pure Sciences and all experiments were carried out in accordance with approved guidelines.

**References**


Nursing Student’s Satisfaction of Nursing Program in the Euphrates

Dr. Hussein Jassim Mohammed¹, R.N Kadhim Hussein Jassim², Salah Saeed³, Mohammed Abass⁴
¹A. Prof. Community Health Nursing, College of Nursing, University of Babylon, ²Babylon Health directorate/ Iraq, ³BSx Nursing graduate, ⁴BSc Nursing graduate.

Abstract

“In higher education, student satisfaction has been viewed as an indicator of program success, and students with higher satisfaction levels progress well in their intellectual and social development” the nursing student satisfaction has not been sufficiently studied in the world. Identifying which factors affect student satisfaction and expectations is valuable to educators seeking to improve the quality of program outcomes.

Cross-sectional survey study of senior nursing students in a BSN program was conducted during Oct 2016-April 2017 at colleges of middle Euphrates, Iraq. (200) senior nursing students in a BSN program from (4) colleges enrolled in the study selected randomly from the last stage of nursing colleges in middle Euphrates. Instruments were developed and used in order to collect data through self-administration of a questionnaire regarding the study, which analyzed by using SPSS, version 23.0. Descriptive statistics (means and standard deviations) were used for demographic questions and for each of the subscales organized by setting. The study results showed highly scaled issue from the participants regarding nursing program satisfaction. college’s recourses had the corner stone of applying nursing education program for nursing students, just (2) items had the high mean of scale (3.06 and 3.22). The study found that all domains were enhancing students satisfaction with their nursing education program.

Key words: Nursing students, nursing program

Introduction

Nursing philosophy has been an associate in Nursing ‘export industry’ since the start of the skilled era in nursing following the Nightingale reforms within the Europe within the Eighties. Nursing education challenges and health care provider education, namely; pair of competencies to patient and population wants, poor cooperation, persistent gender stratification of skilled standing, slender technical focus while not broader discourse understanding, preponderantly hospital orientation at the expense of medical aid and weak leadership to enhance health system performance. Nursing education is incredibly disagreeable with these aspects. Such a disagreeable education could negatively have an effect on a student’s shallowness

Bachelor’s degree nursing program is that the same all universities in Iraq and has undergone varied reforms over the past decades with the intention of making certain that the data and skills of graduates match the necessities for providing top quality care. Challenges were facing nursing education in Asian countries, as well as lack of faculties, learning resources, impassive information and poor collaboration between institutional education strategies and health agency.

Official approval of the nursing program is an important indicator that offers top quality outcomes to meet standards for education in Republic of Iraq hold student satisfaction; nursing student satisfaction is viewed as an associate degree indicator of nursing program success.

In learning action, learner happiness has been viewed as sign of plan achievement, and learner with upper happiness levels movement well in their academic and public increase.

Nursing education has a lot of development achieved in most countries towards building the capability of nursing and midwifery academic establishments in
terms of developing new programmers and change the
nursing and midwifery curricula, particularly in post-
conflict countries. However, the standard of education
in low-income countries needs additional attention,
significantly in relation to investment in infrastructure,
college development and program management.9

The effectiveness, sufficiency, and quality of the
services provided at the nursery, faculties have an effect
on the success of nursing education programs, quality,
and proficiency of nursing students, and the way the
scholars get pleasure from these services are often
measured by their satisfaction.7

Nurses are important resources for achieving health
policy goals and promoting public health, the academic
atmosphere may be a vital indicator in predicting
educational outcome.8

Central to the CFSE(Curriculum, Faculty, Social
Interaction, and Environment)

“Model is the belief that student satisfaction
evolves in a very active method that’s influenced by
the interaction between the scholar and school and
therefore the teaching and learning environments.
During this model,11 projected that the extent of student
satisfaction with a nursing program is directly laid low
with four major constructs: the content and structure of
syllabus, school teaching methods, social interaction
among students and school, and therefore the learning
surroundings, like a nursing skills laboratory”.11

Research Objectives

The aspire of this revise was to give the facts of BSc
nursing course agreement among nursing students using
a regular dimension in order to identify areas needed
for development in general and what is the relation of
characteristic variables and satisfaction

Research questions

What is the satisfaction level of nursing student’s
education?

Methodology

The design of the study: Cross-sectional study
of senior nursing students in a BSc program was
conducted so as to accomplish the stated objectives
during Oct 2016- April 2017 at colleges of middle
Euphrates, Iraq.

Participants: (200) senior nursing students in a
BSc program from (4) colleges enrolled in the study
selected non random from the last stage of nursing
colleges in middle Euphrates, All participants were well-
versed fully about current revise and its aim and then a
charitable oral consent was obtained from the participant
in order to participate in the study. Besides, it has been
taken into account the confidentiality of information
obtained from students.

Instruments: Tool developed and used in order
to collect data. it includes demographic data and four
validated tools assessed satisfaction among nursing
students. For scoring fulfillment status also four point
Likert scales have been used. The items were none or
little of the time, some of the time, a good part of the time
and most or all the time. The four-point Likert scale type
is scored as (1) for none or little of the time, (2) for some
of the time, and (3) for the better part of the time and (4)
for most or all the time. The elevated score means higher
satisfaction.

Data collection: Data were together through self-
running of opinion poll regarding the study. Participants
provide self-report information across fulfillment among
nursing students, with using a developed and well-
constructed opinion poll.

Data analysis: statistics were analyzed using SPSS,
Descriptive statistics (means and standard deviations)
were used for demographic questions and for each
of the subscales organized by setting (classroom or
clinical). Responses were coded on a scale ranging from
1 (disagree) to 3 (agree). Paired t-tests were used to
compare perceived competence in the classroom versus
clinical experiences for each subscale.

Ethical Considerations: Ethical consent was
obtained from a scientific research commute at the
nursing college and governmental health department
(2016/11).
### Results

Table (1) the expressive of teaching and learning domain.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A way that allows me to know the objectives of the program were clarified what should I learn during my studies</td>
<td>3.11</td>
<td>.841</td>
</tr>
<tr>
<td>Application defaulters support students academically methods helped raise the academic level of those who need it</td>
<td>2.30</td>
<td>.971</td>
</tr>
<tr>
<td>Ensure education method in the program many of the activities that have helped me to gain self-learning ability</td>
<td>2.95</td>
<td>.807</td>
</tr>
<tr>
<td>Education style 1 helped me to debate and dialogue to reach the concepts and facts collectively</td>
<td>3.14</td>
<td>.841</td>
</tr>
<tr>
<td>Exact time to acquire skills through practical lessons enough to practice laboratory / field / Applied activities</td>
<td>2.81</td>
<td>.878</td>
</tr>
<tr>
<td>Lots process helped me to develop my abilities in the field of decision V / Profession</td>
<td>3.09</td>
<td>.813</td>
</tr>
<tr>
<td>Field training gave me many of the communication skills and professional skills, according to what has been explained to us</td>
<td>2.74</td>
<td>.985</td>
</tr>
<tr>
<td>I feel after field training has become more confident in my ability to practice the profession and work after graduation</td>
<td>3.01</td>
<td>.808</td>
</tr>
<tr>
<td>Courses taught methods make me feel that I do not need tutoring</td>
<td>2.99</td>
<td>.833</td>
</tr>
<tr>
<td>Availability of faculty office hours at times helped me to understand some of the points that I did not understand during lectures or practical lessons</td>
<td>2.91</td>
<td>1.081</td>
</tr>
</tbody>
</table>

The table indicates certain items with high score regarding nursing program satisfaction at the p value of 0.05.
Table (2) descriptive statistics of student evaluation domain.

<table>
<thead>
<tr>
<th>Description</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calendar varied activities (examinations and extra-curricular activities) to check my abilities and practical Satiable scientific article</td>
<td>3.24</td>
<td>.810</td>
</tr>
<tr>
<td>Calendar-based methods to measure my abilities in knowledge and understanding, interpretation and analysis</td>
<td>3.16</td>
<td>.839</td>
</tr>
<tr>
<td>It is reading the papers my exams quarterly through feedback to learn about my mistakes and learn from them</td>
<td>2.57</td>
<td>1.00</td>
</tr>
<tr>
<td>I find the grades obtained by expressing my level of actual</td>
<td>2.81</td>
<td>.925</td>
</tr>
</tbody>
</table>

Seniors participate in this study failed with satisfaction as student’s evaluation methods in nursing education program.

Table (3) descriptive statistics resources domain.

<table>
<thead>
<tr>
<th>Description</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Educational resources (equipment field / lab / lecture halls, etc.) used to work on the acquisition of professional skills and practical enough</td>
<td>2.62</td>
<td>1.040</td>
</tr>
<tr>
<td>Possibilities for technology sufficient information from my point of view</td>
<td>2.86</td>
<td>.993</td>
</tr>
<tr>
<td>Place in the curriculum, teaching assignments helps me to take advantage of available technology education possibilities and help me with the self-learning process</td>
<td>2.74</td>
<td>.932</td>
</tr>
<tr>
<td>I find that the available scientific literature helps me in the achievement and understanding</td>
<td>3.06</td>
<td>.872</td>
</tr>
<tr>
<td>Teaching aids used in the explanation and clarification will help me a lot to follow and understand the scientific material</td>
<td>3.22</td>
<td>.778</td>
</tr>
</tbody>
</table>

It revealed that just (2) items had the highest mean of the scale (3.06 and 3.22) out of 5.

Table (4) descriptive statistics ensuring fairness and transparency.

<table>
<thead>
<tr>
<th></th>
<th>Description</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I feel confident that the appeal against the results of the examination system</td>
<td>2.36</td>
<td>1.018</td>
</tr>
<tr>
<td>2</td>
<td>I feel justice and equality and the rest of my colleagues interface across all practices that I have taken part</td>
<td>2.59</td>
<td>.920</td>
</tr>
<tr>
<td>3</td>
<td>Tables achieve the greatest flexibility, allowing me to exercise most of the academic activities and the practice of student activities</td>
<td>2.78</td>
<td>.875</td>
</tr>
<tr>
<td>4</td>
<td>I feel that the proposals that extend the overall management and those in charge teaching, be taken into account are studied and utilized</td>
<td>2.36</td>
<td>1.033</td>
</tr>
</tbody>
</table>
Regarding this domain respondent had no significant item.

Table (5) the frequency and percentage of all domains regarding nursing student’s satisfactions. The table indicates (more than 60%) of the nursing students satisfied about their nursing education program.

<table>
<thead>
<tr>
<th>1. Teaching and learning domain</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not Satisfy</td>
<td>17</td>
<td>8.5</td>
</tr>
<tr>
<td>Uncertain</td>
<td>59</td>
<td>29.5</td>
</tr>
<tr>
<td>Satisfy</td>
<td>124</td>
<td>62.0</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>100.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2. Student evaluation domain</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not Satisfy</td>
<td>18</td>
<td>9.0</td>
</tr>
<tr>
<td>Uncertain</td>
<td>58</td>
<td>29.0</td>
</tr>
<tr>
<td>Satisfy</td>
<td>124</td>
<td>62.0</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>100.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>3. Resources domain</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not Satisfy</td>
<td>16</td>
<td>8.0</td>
</tr>
<tr>
<td>Uncertain</td>
<td>56</td>
<td>28.0</td>
</tr>
<tr>
<td>Satisfy</td>
<td>128</td>
<td>64.0</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>100.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>4. Ensuring fairness and transparency domain</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not Satisfy</td>
<td>17</td>
<td>8.5</td>
</tr>
<tr>
<td>Uncertain</td>
<td>59</td>
<td>29.5</td>
</tr>
<tr>
<td>Satisfy</td>
<td>124</td>
<td>62.0</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table (6) nursing student’s had satisfactions about all four domains.

It show that (62.0%) of the participants satisfied in their nursing education program.

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not Satisfy</td>
<td>18</td>
</tr>
<tr>
<td>Uncertain</td>
<td>58</td>
</tr>
<tr>
<td>Satisfy</td>
<td>124</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
</tr>
</tbody>
</table>

About (62.0%) of the participants satisfied their nursing education program.
Discussion

This study agreed with such studies in evaluation of nursing education program, from the student’s point of view, their satisfaction is one methods for evaluation and award accreditation of education in the world, so as, the results of the study

Teaching and learning domain: There were (62.0%) of senior students had satisfied with this domain, in view of the fact that all nursing students from Iraqi universities which had a good history of teaching and learning faculty who were trained in England, as well as had strict rules and regulations in nursing education since 1963.

Kasilingam, et al (2014) who studied this domain and found that a comprehensive assessment of students’ performance within the courses is important in manufacturing graduates who are able to integrate the assumption and apply of the learned courses in some educational activity programs. This issue is important to explain why our student had satisfied, on the other hand, the fact of another researcher about this domain is a positive, high and vital correlation between cognitive-psychomotor learning outcomes and emotive outcomes which often thought to be an indication on the association between these domains (Sonmez, 2017).

Porter, (2006) deliberates that experience with peer ability and education organization strength. The satisfaction level of the good and bad experiences extended towards them by the institute with high student’s satisfactions.

Student evaluation domain: This is the important domain effect on improvement of educational outcome in the higher education, the interpretation of (Remedios and Lieberman, 2008) that “used to determine the manipulation of exams, work, opportunity and objectives on student evaluations of teaching. The results indicated that grades, course complexity, and potentiality did have tiny positive influence on faculty rating, which in turn depended upon the quality of teaching”.

Resources domain: Nursing schools in Iraq supported with enough resources, which reflect the (64.0%) of nursing student that indicate their satisfaction. In “A study of (Dickson, etal, 2015), agreed with the finding and stated that the providing appropriate educational environment, could stimulates students’ thinking and generates their creativity. The results is alike to those of 18, and (Salamonson, et al., 2010), as well as (Wiers-Jenssen, et al. 2002), who found that there is a significant relationship between student academic development (e.g., through intellectually stimulating courses) and overall satisfaction, and a higher satisfaction level when faculty were supposed as sophisticated about the subject matter, set comprehensible goals, and existing quality teaching and a proper workload. The findings are also supported by (Ojeda, et al., 2011), (Row botham, 2010), and (Zafrir and Nissim, 2011), signifying that social relations between faculty and students is fundamental in developing a positive learning environment, which can also lead to student satisfaction. According to AACN’s a statement on 2014-2015 registration and graduation in baccalaureate nursing and postgraduate programs, nursing colleges in the United States turned 68,938 competent applicants of baccalaureate and graduate nursing programs in 2014”.

Conclusion

The study terminates too:

1. Provides student satisfaction of teaching and learning domain, evaluation domain, resources domain, and ensuring fairness as well as intelligibility domain offered in nursing courses which fulfilled within programs in the Middle Euphrates.

2. All domains were enhancing satisfaction for more than half of the respondents, with their nursing education program.

3. The outcome may not be generalized to all nursing programs, or to BSN programs accredited.

4. Nursing faculty members have superior effective teaching in nursing education in spite of their shortage.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Nursing, Iraq and all experiments were carried out in accordance with approved guidelines.

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Relationship Between Nasal Septum Deviation angle and it is Effect on Maxillary Sinus Volume in Sulaimani Population

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²Assist. Prof., Department of Radiology, College of Medicine, Sulaimani University, Sulaimani, Iraq,
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⁴Assist. Lecturer, Department of Radiology, sulaimani polytechnic University, Sulaimani, Iraq

Abstract

Aim of this study: The purpose of this study was to determine the association between nasal septal deviation and the volume of Maxillary sinus in adult population. To analyze the effect of nasal septal deviation on the volume of maxillary sinus. Maxillary sinuses are two spaces, which are filled with air, located in the maxillary bone and can be in various sizes and shapes. Their walls are thin. The apex of the sinuses can extend into the zygomatic process and can occupy the zygomatic bone. We could not demonstrate a statistically significant difference between Groups I–III and Groups II–III, and Group I–II in contra lateral deviation the maxillary sinus volume as the difference were found to be non-significant statistically ($p = 0.965$ and $p = 0.985$, and $p=0.994$ respectively). the mean of (contra lateral side)’s MSV equals to (19.8350) and standard deviation equals to (6.98693) and also the mean of (lateral side)’s MSV equals to (19.5698) and standard deviation equals to (7.69666). Thus, the mean of (contra lateral) for MSV is greater than the mean of (lateral) for MSV. It means that (deviated side) were lower MSV than (contra lateral side) for MSV.

Keywords: Nasal Septum, Effect, Maxillary Sinus Volume

Introduction

The floor formed by the alveolar process, the first, the second and the third molars and the roots of the canines may elevate the sinuses or may perforate their floor. The extent of pneumatisation of the maxillary sinus varies from person to person; its volume is influenced by age, residual dental projections, and alveolar bone height ⁸.

The nasal septum is a midline support structure of the nasal cavity. Deviation of the nasal septum is a common structural cause of nasal obstruction and can arise from dislocation of the quadrangular cartilage from its bony boundaries, or from an intrinsic deformity affecting the vomer, perpendicular plate of ethmoid and/or the quadrilateral cartilage itself ³. Nasal septal deviation can occurred by pressure and expansion during the downward growth of the septum from the ethmoid ossification centers, upward growth of the maxillary crest, and the development of the premaxilla and vomer ⁵. Trauma, particularly which is occurred by injures in infancy and childhood, is a significant factor in the etiology of septal deformity ⁶⁻⁷. The other important etiological factors are irregularity in the growth of the maxilla, asymmetric development of maxillary sinuses and turbinates, thumb-sucking, tongue-pressure habits which cause shifts in the alveolar ridge, genetic and environmental factors ⁵.

High resolution CT of the maxillary sinus has become indispensable in the complete evaluation of the maxillary sinus and its lesions ⁹ and to determine the percentage of pneumatization in the sinus cavity ¹⁰.

Materials and Method

All of the PNS CT scans that were taken in the sulaimania teaching hospital during the last 1 years were used in the study. Head CTs of 50 patients (28 females and 22 males) between 25-30 years’ age. The continuous non-overlapping sections of PNS CT scans were used with acquisition parameters of 1.0-mm slice thickness. The patients with allergic rhinitis, nasal polyposis, cystic fibrosis, asthma, immune deficiency, malignancy, metabolic disease, and those previous underwent maxillofacial
trauma, sinus surgery were excluded in this study.

**Measurement (done by CT scan)**

The nasal septal angle (NSA) was measured as the angle between a line drawn from the superior insertion of the nasal septum at the crista galli to the inferior insertion of the septum at the maxillary crest and another line from the superior insertion of the septum at the crista galli to the maximally deviated point of the convex nasal septum. Patients with NSD were grouped into 3 groups according to the measured nasal septal angle as group I (mild, $<9^\circ$), group II (moderate, $\geq 9^\circ$ and $<15^\circ$), or group III (severe, $\geq 15^\circ$), according to Elahi et al.’s grading system.  

**Measurement of maxillary sinus volume**

Maxillary sinus volume is measure by following equation.

\[
\text{Volume} = (\text{height} \times \text{depth} \times \text{width} \times 0.5).
\]

**Statistical Analysis**

The data were analyzed using IBM SPSS software (version 22, International Business Machines Corp., Armonk, NY, USA), and $p <0.05$ was considered statistically significant.

**Result and discussion:**

A total of 50 young adult, consisting of 31 right-sided and 29 left-sided NSDs, were included in the study. There were 22 males and 28 females, whose mean age was $30.38\pm3.658$ years, ranging from 25 to 35 years. Mean NSA was $10.15\pm4.85$ degrees, ranging from 3.8 to 24.5 degrees.

The deviation angles varied between $5^\circ$ and $24^\circ$. The mild group had 25 cases, the moderate group had 17 cases, and the severe group had 8 cases.

We could not demonstrate a statistically significant difference between Groups I–III and Groups II–III, and Group I–II in contra laterally deviated the maxillary sinus volume as the difference were found to be non-significant statistically ($p = 0.965$ and $p = 0.985$, and $p=0.994$ respectively).

Maxillary sinus volume in deviated side were Groups I and II and Groups I–III and Groups II–III compartments ($p = 0.565$, $p = 0.895$ and $p = 0.944$, respectively). Table 1.(11,12)

**Table 1: statistical description of maxillary sinus volume between each groups with (p value) by using T- test.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I V Group II</td>
<td>0.994</td>
</tr>
<tr>
<td>Group III V Group II</td>
<td>0.985</td>
</tr>
<tr>
<td>Group I V Group III</td>
<td>0.965</td>
</tr>
</tbody>
</table>

**Table 2: : statistical description size of the MSV between (contra lateral and lateral)**

<table>
<thead>
<tr>
<th>MSV</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>con contra later side</td>
<td>25</td>
<td>19.835</td>
<td>6.98693</td>
</tr>
<tr>
<td>deviated</td>
<td>25</td>
<td>19.5698</td>
<td>7.69666</td>
</tr>
</tbody>
</table>

It can be indicated in the table (2) that the mean of (contra lateral side)’s MSV equals to (19.8350) and standard deviation equals to (6.98693) and also the mean of (lateral side)’s MSV equals to (19.5698) and standard deviation equals to (7.69666). Thus, the mean of (contra lateral) for MSV is greater than the mean of (lateral) for MSV. It means that (deviated side) were lower MSV than (contra lateral side) for MSV.
### Table 3: statistical description a relationship between contra lateral side and deviated side

<table>
<thead>
<tr>
<th>Paired Samples Correlations</th>
<th>contra lateral side</th>
<th>Deviated side</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Correlation</td>
<td>0.758</td>
</tr>
<tr>
<td></td>
<td>Sig.</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Sample</td>
<td>50</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Deviated side</th>
<th>Correlation</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sig.</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Sample</td>
<td>50</td>
</tr>
</tbody>
</table>

The level of significance at level 0.05  
There is a relationship between the statistical function between the contra lateral and latral

It is noted from Table (3) that there is a statistically significant positive correlation between (A relationship between the contra lateral side and deviated side) which is (0.758) and that the significance value is 0.000 and is less than 0.05. This indicates acceptance of the second hypothesis, A positive correlation between the extent of (A relationship between the contra lateral side and deviate side)\(^{(13,14,15)}\).

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of dentistry, Iraq and all experiments were carried out in accordance with approved guidelines.

**Reference**


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Detection of the vaginal microflora in Pregnant and non-pregnant women using culture-independent method

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Abstract

Background: Bacterial Vaginosis (BV) is a disorder characterized by alterations of the vaginal flora with acquisition of diverse communities of anaerobic and facultative bacteria and depletion of the usually dominant lactobacilli.

Objective: To conduct and characterize the main bacterial players in BV affected pregnant and non-pregnant women.

Method: The BV work included collecting vaginal swabs from 158 women (18-45) years old. The samples were subjected in a laboratory to Amsel’s clinical criteria (ACC) and Nugent score system (NSS). The clinical diagnosis of BV was comprehensive when at least three ACCs were present out of a total four criteria (pH of vaginal secretions (Vss), Vaginal discharge, Clue cells and Wiff (sniff) test).

Results: The findings of the ACC and NSS revealed BV in 32 pregnant women (Pw) and 55 non-pregnant women (Npw). In detail, the ACC identified 39 (15 Pw and 24 Npw) positive samples. On the other hand, NSS unveiled that 48 (17 Pw and 31 Npw) with Vss were positive to BV. The bacterial isolates were distributed over the age categories (18-47 years old) of the Pw and Npw, in all age categories, (18-23, 24-29, 30-35, 36-41, and 42-47) year old, respectively.

Conclusion: The presented work, here, provides beneficial data obtained by using feasible techniques with high degrees of reliability to overcome setbacks generated from the use of cultivation techniques.

Keywords: vaginal microflora, Pregnant, Non-pregnant.

Introduction

Bacterial Vaginosis cause of foul vaginal discharge and is linked to the number of horrible health outcomes in reproductive age of females since has been associated with serious clinical obstetrical and gynecological sequelae, of these are infertility, endometritis, pelvic inflammatory disease (PID), post-abortion sepsis, post-surgical abortion infections, post-hysterectomy infection, and increased risk of HIV and other STIs acquisition¹.

BV is a clinically well-understood health condition manifested by the presence of a pH ≥ 4.5-vaginal discharge with properties of fishy-odor, gray to white, and homogenized adherent thin smear which is highly recognized after intercourse and menses². BV is the most prevalent vaginal disease that happens when useful Lactobacillus spp. decreases and different obligated or facultative anaerobic VBS elevated, which were usually available in very small amounts or absent in non-BV females³. This dysbiosis induces he BV pathological processes predominated by vaginal discharge which is an indicative sign for BV in 20 to 30% of females; however, risk can be higher as 50–60% in some active communities sexual behaviors (4,5).

Culture-dependent methods for BV identification face potential obstacles represented by non-cultivability of a wide range of bacterial members which may affect the reality of the profile of BV causative agents and the functions of bacterial members⁴.
Ecosystem of the vagina include several aerobic and anaerobic microorganisms coexist in a dynamic balance, this homeostasis results from complex interactions and synergies among the host and different microorganisms that colonize the vaginal mucosa.

Studies conducted in Iraq revealed that BV rates were between 28% and 37.5% among both pregnant and non-pregnant women.

The present BV-directed work was conducted aiming at understanding the real bacterial composition of the vaginal tract in pregnant and non-pregnant women (Pw and Npw, respectively) at reproductive age using primary scoring criteria non-cultivating.

Materials and Method

Subjects:

A total of 158 females, aged from (18-45) years old who visiting the outpatient clinics in the Maternity and Pediatrics Teaching Hospital during a period from November 2018 to April 2019 in Al-Diwaniya city, to evaluate of vaginal flora between women with and without bacterial vaginosis. Any participant having any of the following exclusion criteria was excluded from participation: <18 years of age, Diabetes mellitus, Using of antimicrobial agents (orally or suppositories), Females during menses period and Females using intrauterine contraceptives.

Samples collection and preparation:

By guidance of gynecologists, a sterile unlubricated speculum was inserted into the vagina and specimens were collected from the vaginal wall lateral areas and posterior fornix using sterile cotton tipped swabs and were carefully removed to avoid contamination with microflora of the vagina. The swabs were Amies-transport-media inserted and then transported to the designated facility, two swabs were taken from each women were used for gram stain preparation.

Amsel’s Criteria

The clinical diagnosis of BV was comprehensive when at least three ACCs were present out of a total four criteria. These criteria are as follows:

- **PH of vaginal secretions:** With a cotton swab the vaginal discharge were collected then transferred on a narrow range (3.5-6.0) PH strips for determined the PH directly.

- **Vaginal discharge:** During pelvic examination the clinician will evaluate the nature of the vaginal discharge, in BV case the discharge have to be thin, homogenous, and with milky colour.

- **Clue cells:** A high vaginal swab was used to prepare a dry vaginal smear by rolling it along the middle of a glass slide, the smear was air-dried and fixed with methanol then stained by Gram stain. The epithelial cells will be completely covered by the gram variable coccobacilli with indistinct borders due to adherent bacteria.

- **Wiff (sniff) test:** Adropletof 10% potassium hydroxide (KOH) was added to the vaginal discharge on a glass slide and immediately evaluated for the presence of a fishy smell which reveals a positive outcome.

Nugent’s Criteria and Gram Staining

The stained slides were examined under oil immersion objective 1000x magnification and evaluated for the following morphotypes: *Lactobacillus* morphotypes; longrods with Gram-+ve criteria, *G. vaginalis* morphotypes; small rods with Gram-±ve, *Bacteroides* spp. morphotypes; small rods with Gram–ve properties, and *Mobiluncus* spp. morphotypes; small curved rods with Gram–velooking. Morphotypes were counted separately from 0 to 4+ in respect to the single-oil-immersion field morphotype numbers in which 0:nil morphotypes, 1+:˂1 morphotype; 2+: 1-5 morphotypes, 3+: 5-30 morphotypes, and 4+:˃30 morphotypes. These scores added up to yield a final score of (0-7) or more. The score (0-3) is considered as normal, (4-6) is considered intermediate, and the condition of BV is a score of (7-10).

Results & Discussion

Although many BV-directed studies have been done, the picture of knowledge about the real vaginal bacterial composition in BV- or non-BV-affected women is not completely clear. Those studies either were focused on determining the major bacterial players in BV or characterizing the BV as a health condition with multiple bacterial members.

High vaginal swabs were collected from 158 women at different reproductive age groups, (18-45) years old, a total of (53) pregnant women who had abnormal vaginal discharge during the study period.
were recruited. Further than these women with atypical vaginal discharge, and (105)samples were taken from non-
pregnant women complaints of abnormal vaginal discharge, odor, and itching or burning (Figure 1).

![Pie chart showing the distribution of bacterial vaginosis among pregnant and non-pregnant women. The chart indicates that 33.54% of pregnant women and 66.45% of non-pregnant women had bacterial vaginosis.]

Figure (1): Distribution of bacterial vaginosis according to pregnant and non-pregnant women.

Thirty two pregnant women had bacterial vaginosis giving an occurrence rate of (36.78%) these women were positive for bacterial vaginosis using both the clinical criteria and gram stain morphology, 15 (38.46%) were positive by using the Amsels clinical criteria alone while 17 (35.41%) pregnant women had bacterial vaginosis using the Nugent score system only as shown in Table (1), Table (2).

Table (1) the Nugent’s score system

<table>
<thead>
<tr>
<th>Score</th>
<th>Morphotype of Lactobacillus spp.</th>
<th>Morphotype of Gardnerella and Bacteroides spp.</th>
<th>Morphotype of curved bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>3+</td>
<td>1+</td>
<td>1+ or 2+</td>
</tr>
<tr>
<td>2</td>
<td>2+</td>
<td>2+</td>
<td>3+ or 4+</td>
</tr>
<tr>
<td>3</td>
<td>1+</td>
<td>3+</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>4+</td>
<td></td>
</tr>
</tbody>
</table>

Table (2): Occurrence of bacterial vaginosis in pregnant and non-pregnant women using culture-independent method.

<table>
<thead>
<tr>
<th>Diagnostic tool</th>
<th>Total number</th>
<th>Positive number</th>
<th>Pregnant</th>
<th>Non-pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amsels clinical criteria</td>
<td>158</td>
<td>39(24.68)</td>
<td>15(38.46)</td>
<td>24(61.53)</td>
</tr>
<tr>
<td>Nugent score system</td>
<td>48(30.37)</td>
<td>17(35.41)</td>
<td>31(64.58)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>-----</td>
<td>87</td>
<td>32(36.78)</td>
<td>55(63.21)</td>
</tr>
<tr>
<td>X2</td>
<td>1.285(NS)</td>
<td>0.086(NS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.257</td>
<td>0.770</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS: Non-significant difference at p ≥ 0.05
The incidence rate of BV among pregnant women in this study (36.78%) falls within the range of previous studies (17.3 – 67.7%) but is higher than that of another studies which ranged from (3.54 – 25.1%) considering local studies on the subject, the occurrence of (36.78%) is higher than the results reported by Al-Fadul were (13.1%) in pregnant women only.

This differences could be related to the different populations studied and difference in the gestation period, the present study involved pregnant women who presented at reservation during the study period regardless of gestational age, or whether they were symptomatic or not at acceptance.

Nevertheless, bacterial vaginosis incidence during pregnancy has an importance since it was revealed to be related with numerous obstetric sequalae such as preterm labor, still births, postpartum infections, premature rupture of membranes, abortion and low weight infants.

Fifty five non-pregnant women had bacterial vaginosis giving an occurrence rate of (63.21%) these women were positive for bacterial vaginosis using both the gram stain morphology, and Amsels clinical criteria, whereas 31 (64.58%) non-pregnant women had bacterial vaginosis using the Nugent score system (Table 1).

The incidence rate of (63.21%) among non-pregnant women is lower than the reported rate in a previous study were conducted in Iraq (86.9%) (Al-Fadul et al., 2007), but with higher rate reported in a foreign study in Egypt (33%) . Socio-demographic characteristics, sexual activity, reproductive health information, and behavioral or genital hygiene have been identified as causes of variation in the prevalence rates of bacterial vaginosis among non-pregnant women.

In our study, the results of the Amsel’s test shown that 39 (24.68%) subject of infected women gave positive results (15 were pregnant women and twenty four were non-pregnant women) since they matched three of four Amsel's clinical criteria. The most significant criteria for diagnosis of BV are as follows: presence of a thin homogenous discharge, a pH of vaginal fluid greater than 4.5 and a positive KOH 10% test. (Wiff test). The attendance of clue cells in the wet smear upon microscopic examination was reflected, clue cells are a critical component of Amsel’s criteria for BV diagnosis.

The use of Amsels clinical criteria to diagnose BV has the advantage of rapid diagnosis at the point of care but requires assessment of vaginal pH and, more importantly, performance of microscopy of vaginal fluid by a sensibly skilled consultant or laboratory personnel.
The results of vaginal discharge inspection is homogenous, thin, milky and adherent to the vaginal wall that exposed by clinicians or physician women help.

Definitely there is a diverse in the talent of clinicians to remark the vaginal discharge, abnormal discharge commonly associated with other vaginal infections rather than BV such as trichomoniasis and candidiasis, conversely presence of vaginal fluid may be enhanced by numerous factors as well as sexual intercourse, menstruation and douching.

In the present study vaginal pH was measured by using narrow range pH papers, by which the pH value is visually examined through the estimate of the test strip to a standard colour scale with fixed values, i.e. pH persistence rely on the examiner, Hence, there is no specific interpretation number for pH.

Wiff test were used in this study for detection of fishy odor associated with BV, and this test like other Amsel’s criteria, is also subjective and depends on the investigator variation in the ability to detect the characteristic amine odor, infection with T. vaginalis may give positive result for Wiff test, also false positive KOH test can occur in women whose have had sexual intercourse recently, finally, when Wiff test gives a positive result, the sample become without amine odor due to volatility of amines quickly and completely.

The presence of clue cells in stained smears was the most definite criterion among the other Amsels clinical criteria and they have not been detected in any subject without BV.

The overall occurrence rate of bacterial vaginosis in the present study as determined by Gram-stain Nugent scoring criteria was (30.37%), the reported rate of BV was well within the reported range, (8%–75%) in some studies being conducted in Iraq, the occurrence rate of bacterial vaginosis ranged from (37.5%) to (68.7%) by using Nugent's scores as adiagnostic tool (8), but it’s with higher rate reported by another study in India (24.3%).

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Medicine, Iraq and all experiments were carried out in accordance with approved guidelines.

References
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Detection of **mec A**, **van A** and **van B** genes of *Staphylococcus aureus* Isolated from Patients in Al Muthanna Province Hospitals

Adnan H.Aubaid 1, Ziaoon H. Mahdi 2, Tabarek S. Abd-Alraoof 2, Noor M.Jabbar 2

1Prof. Dr., 2MSc. Student, Department of Medical Microbiology, College of Medicine, Al-Qadisiyah University, Diwaniyah, Iraq

Abstract

**Background:** Infections triggered by methicillin-resistant *Staphylococcus aureus* (MRSA) and onset Resistance to significant anxiety in healthcare environments worldwide from vancomycin-resistant *Staphylococcus aureus* (VRSA).

**Aim:** To discover *Staphylococcus aureus* resistant to methacillin and vancomycin by detecting (Mec A, Van A and Van B) genes by PCR technique.

**Methods:** A total of 250 samples from patients with different clinical cases whom admitted to Hospitals in Al Muthanna province during a period from January 2019 to July 2019. *Staphylococcus aureus* were isolated and identified by using cultural and biochemical tests. The extracted DNA of isolates were amplified by PCR to detect (mecA, vanA, and vanB) genes.

**Results:** The results showed that 72/ 250 of *Staphylococcus aureus* isolates contained mecA gene, indicating that all isolates are Methicillin resistant *S. aureus* (MRSA), only five isolates contained van A gene and only nine isolates contained van B gene.

**Conclusion:** Appropriate monitoring and control measures appear to be crucial to avoid the development and transmission of MRSA and VRSA strains in our nation.

**Key words:** Methicillin resistant Staphylococcus aureus(MRSA); vancomycin resistant Staphylococcus aureus(VRSA); mec A, vanA ,vanB genes.

Introduction

*Staphylococcus aureus* is a human pathogen as well as a commensal bacterium. *Staphylococcus aureus* colonizes about 30 percent of the human population. At the same time, it is a major cause of bacteremia and endocarditis (IE) and osteoarticular, skin and soft tissue, pleuropulmonary, and devicerelated infections. Horizontal transfer of antibiotic resistance genes and production of multiple virulence factors are thought to play a major role in the emergence and persistence of resistant *S. aureus* strains in the hospital environment. *Staphylococcus aureus* possesses several virulence factors that contribute to colonization and invasion of host tissue, evasion of the hosts’ immune systems, and nutrient acquisition. Methicillin resistant *S. aureus* (MRSA) outbreaks have become a serious problem in healthcare settings worldwide. The therapeutic options in MRSA infections are limited to glycopeptides, linezolid, tigecycline, and ceftaroline. Methicillin resistance is mediated by mecA and acquired by horizontal transfer of a mobile genetic element designated staphylococcal cassette chromosome mec (SCCMec). The gene mecA encodes penicillin-binding protein 2a (PBP2a), an enzyme responsible for crosslinking the peptidoglycans.
in the bacterial cell wall. PBP2a has a low affinity for β-lactams, resulting in resistance to this entire class of antibiotics. In addition to the mecA gene, S. aureus carries the mecA regulatory genes mecI and mecR1, which are divergently transcribed regulatory genes located immediately upstream from the mecA promoter. They are similar in molecular organization, structure, function, and mechanism of regulation to Staphylococcal β-lactamase regulatory elements, blaI and blaR1.

Five different types of SCC mec elements, types I to V, have been identified. The type I SCC mec contains the mecA gene as the only resistance element, while the type II and III elements contain, besides mecA, multiple determinants for resistance against non-β-lactam antibiotics. Accordingly, type II and III SCC mec elements are responsible for multidrug resistance in nosocomial MRSA isolates. Type IV SCC mec elements, like type I elements, contain no resistance genes other than mecA, and they are significantly smaller than the type II and III elements. This might serve as an evolutionary advantage, making it easier for these mobile genetic elements to spread across bacterial populations.

During the last decade, S. aureus isolates with decreased susceptibility to vancomycin (vancomycin intermediate S. aureus (VISA) and vancomycin resistant S. aureus (VRSA) have been reported in various parts of the world. These isolates are associated with increased mortality because of the limited therapeutic options remaining.

Vancomycin is a glycopeptide and is very popular and competent antimicrobial drug for treating MRSA infections but unfortunately resistance to vancomycin have also been reported since 1997. This vancomycin resistance is actually due to the presence of Van resistance genes which are acquired from enterococci and are encoded on R-plasmid or chromosome. There are total six genes responsible for vancomycin pyruvate into D-Lactate, ligase that synthesizes D-Alanyl–D-Lactate and a dipeptidase that hydrolyzes D-Alanyl–D-Alanine. The collective action of these three enzymes incorporate DAAlanyl–D-Lactate instead of D-Alanyl–D-Alanine into peptidoglycan which prevents the attachment of vancomycin. The vanA gene contributes higher resistance to vancomycin and teicoplanin both glycopeptides while vanB gene which is also an effective resistant gene encodes resistance to vancomycin only. The vanB gene resistance which includes vanA, vanB, vanC, vanD, vanE and vanG whereas vanA and vanB genes are the most important and prevalent genes.

The examples of resistant strains are vancomycin intermediate S. aureus (VISA), vancomycin resistant S. aureus (VRSA) and heterogeneous VISA (hVISA) strains. The mechanism of resistance for both vanA and vanB genes encompass three sets of enzymes harbored by glycopeptide-producing bacteria. The enzymes are dehydrogenase that reduces clusters (vanB1, vanB2, and vanB3) are generally carried by large elements (90-250 kb) which are transferable by conjugation from one chromosome to another and often contain the transposon Tn1547. Studies have also been conducted on detection of vanB gene in clinical isolates of Staphylococcus aureus strains but a few cases have been reported so far.

**Materials and Method**

A total of 250 samples from different clinical cases (wound, urine, high vaginal, throat, skin, ear, nose and pus swabs) were taken from patients of different ages and gender whom were admitted to Al Hussein Educational Hospital, Children’s Hospital and the women’s Educational, Rumaita General Hospital, El Khidhir General Hospital and Public Health Laboratory in Al Muthanna province. The samples were collected during a period from January 2019 to July 2019. only 72 samples were identified as Staphylococcus aureus by using cultural and biochemical testes.

Genomic DNA was extracted from bacterial isolates by using Genomic DNA Mini Bacteria Kit. The extracted DNA was checked by using Nanodrop (THERMO. USA) that measured DNA concentration (ng/µL) and checked the DNA purity by reading the absorbance at (260 /280 nm). Polymerase chain reaction master mix for each gene was prepared by using (Maxime PCR PreMix kit) and this master mix done according to company instructions. Polymerase chain reaction assay was performed for detection antibiotic resistance genes (mecA, vanA, and vanB gene) in Staphylococcus aureus. Table (1).
Table (1): The PCR primers for *Staphylococcus aurous* antibiotic resistance genes

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence 5'-3'</th>
<th>Amplicon</th>
</tr>
</thead>
<tbody>
<tr>
<td>mecA gene</td>
<td>F GTGAAGATATACCAAGTGATT</td>
<td>147bp</td>
</tr>
<tr>
<td></td>
<td>R ATGCCTATAGATTGAAAGGAT</td>
<td></td>
</tr>
<tr>
<td>vanA gene</td>
<td>F GGCAAGTCAGGTGAAGATG</td>
<td>713bp</td>
</tr>
<tr>
<td></td>
<td>R ATCAAGCGGTCATACGTTC</td>
<td></td>
</tr>
<tr>
<td>vanB gene</td>
<td>F GTGACAAACCGGAGGCGAGGA</td>
<td>430bp</td>
</tr>
<tr>
<td></td>
<td>R CCGCATCCTCTCGCAAAAA</td>
<td></td>
</tr>
</tbody>
</table>

Then the Polymerase chain reaction products were visualized in an ethidium bromide-stained 1% agarose gel using a UV Transilluminator.

**Statistical Analysis**

All the results of the present study were analyzed statistically by Social Science Statistics and the Statistical Package For Social Sciences version 23 for Windows Software (Inc., Chicago, IL, USA). Chi-square test (X²) was used for the assessment of differences between the variables studied. The P values less than 0.05 were considered statistically significant and high significant respectively. (14,15).

**Results and Discussion**

**Bacterial Isolation & Identification**

The present study included 72 isolates from *Staphylococcus aureus* which isolated from Urine, High Vaginal Swab, Pus, Wound, Nose, Ear, Throat and Skin Swab. All isolates had the ability to grow on the mannitol salt agar which considered selective and differential media for genus *Staphylococcus* (16). The colonies appeared round, smooth, raised, mucoid and shiny. Consequently, the isolates belong to the genus Staphylococcus.

All isolates had the ability to ferment mannitol and form large golden colonies surrounded by wide yellow zones and turned the colour of the medium from pink to yellow as showed in figure (1).

![Figure 1: Mannitol salt agar medium(A) colonies of *Staphylococcus aureus* ferment mannitol and form large golden colonies surrounded by wide yellow zones (B).](image)

Microscopic examination was applied to the all 72 isolates after staining by Gram stain and the cells appeared as Gram-positive cocci arranged in grape-like irregular cluster. For further identification, the catalase test performed and all 72 isolates gave positive results. A coagulation test was performed to identify bacterial isolates at the species level, and all isolates showed the ability to produce coagulation. Isolates examined for colony characterization after sub culturing on 5% human blood agar and incubated for 24 hrs. at 37°C. The colonies appeared large, round, golden colony surrounded with a halo clear zone of hemolysis. It is often β-hemolysis type on blood agar.

**Screening for resistant genes by Polymerase chain reaction**
Polymerase chain reaction (PCR) was performed for 72 isolates *Staphylococcus aureus* to detect the presence of *mecA* genes (figure 2). The results showed that all 72 isolates contained *mecA* gene, indicating that all isolates are resistant to antibiotic Methicillin (MRSA).

![Figure 2](image)

Figure (2): Agarose gel electrophoresis image that showed PCR product analysis for methicillin antibiotic resistance *mecA* gene in *Staphylococcus aureus* isolates. M (Marker ladder 1500-100bp). Lane (1-10) some positive *mecA* gene *Staphylococcus aureus* isolates at 147bp product size.

Polymerase chain reaction (PCR) was performed for all 72 isolates of *Staphylococcus aureus* to detect the presence of *vanA* gene (figure 3). The results showed that only five isolates contained *vanA* gene, indicating that only five isolates are resistant to antibiotic vancomycin.

![Figure 3](image)

Figure (3): Agarose gel electrophoresis image that showed PCR product analysis for vancomycin antibiotic resistance *vanA* gene in *Staphylococcus aureus* isolates. M (Marker ladder 1500-100bp). Lane (1-4) only positive *vanA* gene *Staphylococcus aureus* isolates at 713bp product size.
Polymerase chain reaction (PCR) was performed for all (72) isolates *Staphylococcus aureus* to detect the presence of van B gene figure(4). The results showed that only nine isolates contained van B gene, this indicate that only nine isolates are resistant to antibiotic Vancomycin

![Agarose gel electrophoresis image](image)

**Figure (4):** Agarose gel electrophoresis image that showed PCR product analysis for vancomycin antibiotic resistance vanB gene in *Staphylococcus aureus* isolates. M (Marker ladder 1500-100bp). Lane (1-7) only positive vanB gene *Staphylococcus aureus* isolates at 430bp product size.

This genetic study included Polymerase chain reaction (PCR) assessment of mecA, vanA and vanB over expression and the results showed that mecA gene was over expressed in 72 (100 %) isolates, vanA gene was only expressed in 5 (6.9 %) and vanB was only expressed in 9 (12.5 %) isolates, as shown in Figure (5).

![Distribution of genes](image)

**Figure( 5):** Distribution of mecA, vanA and vanB genes in *Staphylococcus aureus* isolates.

The results showed that all isolates of *Staphylococcus aureus* are contain mecA gene, this means that all isolates were methicillin resistance *Staphylococcus aureus* (MRSA), our study approach with 17 in Iran, reported that all 46 MRSA isolates harbored mecA gene. However we disagree with 18 in Qadisiyah, who showed that (19%) of isolates were have mecA gene methicillin resistance. 19 in Bangladesh, was disagree with this study, they suggest that prevalence of mecA in the isolates was 72%.
The results showed that five isolates of *Staphylococcus aureus* are contain vanA gene, and this means only (6.9%) isolates were Vancomycin resistance (VRSA) and this approach came with 20 in Egypt , who reported 20.13% of the VRSA emerged contain vanA gene. Also Fasihi et al. 21 in Iran, agreed with the present study, they found that two MRSA isolates were identified as VRSA and both isolates were vanA gene positive.In addition we agree with 22 in Iran, they report that vanA gene was found in two VRSA isolates. However we disagree with the results of 23 in Egypt, they reported the occurrence of vanA gene-negative VRSA in Egypt. Other study done by 24 in Pakistan,who disagree with our results ,they found no vancomycin resistant strain in their setup.

Our results showed that nine isolates of *Staphylococcus aureus* are contain vanB gene, this mean only (12.5 %) isolates were Vancomycin resistance (VRSA) and approach came with 25 in sudan , reported that only three VRSA contained vanB. Also 26 in Bangladesh , found that VRSA strains were positive for the vanB gene but negative for the vanA gene. Although our result disagree with 22 in Iran, who showed that vanB gene was not detected in any VRSA isolates. El-Banna et al. 20 in Egypt, described that VanB gene were not found in any isolates.In conclusion, the *Staphylococcus aureus* isolates have mec A gene, which is characteristic of Methicillin-resistant *Staphylococcus aureus* (MRSA), and only five isolates on the van A gene is proof that they are resistant to vancomycin *S .aureus* (VRSA). Also, only nine isolates possessed the van B gene and are evidence of vancomycin resistance *S .aureus* (VRSA).

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of Medicine, Iraq and all experiments were carried out in accordance with approved guidelines.

**References**


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Client’s Attitude Regarding Antibiotics Misuse in Primary Health Care Centers

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Abstract

Antibiotics are powerful drugs that fight some infections and can save lives when used properly. It either stops the bacteria from multiplying or destroying them.

Objectives: The study aims to: 1. Evaluate the client’s position regarding the abuse of antibiotics in primary health care centers. 2. Identification of relationships of abuse of antibiotics and social demographics (age, sex, place of residence, level of education, economic status, profession, marital status).

Methods: A descriptive study (quantitative design) was carried out using a cross-sectional design to study the assessment of the client’s position on the misuse of antibiotics in primary health care centers in Diwaniya from July 2018 to July 2019. A sample of 450 female clients and 221 women in primary health care centers in Diwaniyah (Al Jazira Health Center, Al Talea Health Center, Al Taqia Health Center, New Health Center, Al Furat Health Center) The questionnaire was created through a comprehensive review of available literature and related studies.

Results: The results of the study show that the clients do not agree with the negative attitude regarding the misuse of antibiotics. Therefore, the study reveals a relationship between the social demographic and the second part. The result shows that there is a very large relationship between all elements of the second part with age, Educational level, economic status, occupation, social status. While there is little correlation with gender and place of residence.

Keywords: Client’s Attitude, Antibiotics, Primary Health Care Centers

Introduction

Antibiotics are powerful medicines that fight certain infections and can save lives when used properly. They either stop bacteria from reproducing or destroy them. The first antibiotic was penicillin. There are several types of modern antibiotics, and they are usually only available with a prescription in most countries. Topical antibiotics are available in over-the-counter creams and ointments (OTC). There are different types of antibiotic, which work in one of two ways: A bactericidal antibiotic, such as penicillin, kills the bacteria. A bacteriostatic stops bacterium from multiplying.

Antibiotic resistance occurs when bacteria change in some way that reduces or eliminates the effectiveness of drugs, chemicals or other agents designed to cure or prevent the infection. Thus, the bacteria survive and continue to multiply causing more harm. Widespread use of antibiotics promotes the spread of antibiotic resistance (Lu, L., and Gao, M., 2018). Several studies conducted across the globe in low, middle- and high-income countries have revealed inappropriate antibiotic use, which correlates positively with antibiotic resistance development (Rather, I. A., 2017). Antibiotics are appearing to be used not only in excess but also inappropriately and this accounts for 20% to 50% of all antibiotics used (Porter, G., and Grills, N., 2015).

Literature Review

Study’s Imogene King’s goal attainment theory was used as conceptual framework. She defined nursing as “a process of action, reaction and interaction by which nurse and client share information about their perception in a nursing situation” and “a process of human interactions.
between nurse and client whereby each perceives the other and the situation, and through communication, they set goals, explore means, and agree on means to achieve goals (Alligood, M. R., 2017).

Antibiotics are drugs used for treating infections caused by bacteria. Also known as antimicrobial drugs, antibiotics have saved countless lives. Misuse and overuse of these drugs, however, have contributed to a phenomenon known as antibiotic resistance. This resistance develops when potentially harmful bacteria change in a way that reduces or eliminates the effectiveness of antibiotics (Cantavella J., and Martínez, A., 2011).

The six main causes of antibiotic resistance have been linked to: Over-prescription of antibiotics, Patients not finishing the entire antibiotic course, overuse of antibiotics, Poor infection control in health care settings, Poor hygiene and sanitation Absence of new antibiotics being discovered. (Mendelson, M., and Matsoso, M. P., 2015)

### Methodology

#### Design of the Study:

A descriptive study was carried out by using a cross-sectional design to study Client’s Attitude Regarding Antibiotics Misuse in AL-Diwaniya City’s Primary Health Care Centers from July 2018 up to July 2019.

#### Setting of the Study:

The study was conducted primary health care centers in AL-Diwaniya City.

AL- Diwaniya city is one of the rich cities in health care facilities; it contains many primary health care centers (AL- Jazaer Health Center, Al - Tali’ah Health Center, AL-Taqiya Health Center, AL- Jadera Health Center, and Al Furat Health Center).

#### Reliability of the Study Instrument:

To identify the reliability of the study instrument, Alpha Correlation Coefficient (R) was applied for the purpose of measuring the internal consistency of the study instrument by applying of Statistical Package for Social Science Program (IBM SPSS) version 20.0.

#### Table (1): Reliability of pilot study

<table>
<thead>
<tr>
<th>Reliability</th>
<th>Client’s Attitude Regarding Antibiotic Misuse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha Correlation (r)</td>
<td>0.80</td>
</tr>
</tbody>
</table>

#### 3.10. Data Collection

The researcher obtained data from (450) samples of clients within five primary health care centers (AL-Jazaer Health Center, Al - Tali’ah Health Center, AL-Taqiya Health Center, AL- Jadera Health Center, Al Furat Health Center) by using a constructive questionnaire format that was answered through self-report.

### Results

### Discussion

The result of this study shows the majority (39.6%) of the present study are in the last week taking antibiotics, ( 71.6%) of the present study are get the antibiotics prescription from a doctor, (46.0%) of the present study are get the antibiotics prescription from a nurse, (72.9%) of the present study are received advice from a doctor or nurse on how to take antibiotics for 7 days, (70.0%) of the present study are get the antibiotics from Pharmacy, (57.3) of the present study are stop taking antibiotics when you feel better.

These finding agree with the result of 2 who reported that (62.1 %) of the respondents took medication by consulting a physician, and disagree with the same previous study who shown that (17.3%) of the respondents get antibiotics and advice from pharmacist, followed by the finding disagree with the obvious study where 8% of them seeking the advice of family/friend member regarding the source of antibiotics.

The finding is supported by the result of Nawafleh, H., et al., (2016) who reported in their study that the majority of participants (33.7%) were getting information from physician about using the antibiotics, and disagree with the same previous study who stated that (18.2%) of the respondents get antibiotics and advice from pharmacist, while (15.2%) of them get from the Previous experience. But the finding agreed with previous study who shown that (10.4%) of participants getting information from friends.
The result of this study shown that the most of client’s answers are yes in all items of this part, where the item number (4) (level of education can contribute to the misuse of antibiotics especially, patients in our community), this item formed the high percentage (54.2) than the other items in this part. While the items number (9,15) (Patients not finishing the entire antibiotic course— is a major cause of antibiotic resistance, Primary reason for people to indulge in self-medication is the expensive healthcare system) formed the low percentage (50.4) than the other items in this part.

**Conclusion**

Based on the study results, the researcher concludes the following:

- Most of clients were elementary school graduate according to level of education, Regarding place of residence, most of clients (54.7%) were urban
- Most of the clients who misused the antibiotics received it from the pharmacy with an old medical recipe.
- Doctors and pharmacists don’t devote enough time to explain the treatment and side effects.
- The absence of counseling, educational courses and posters by health institutions is one of the most important factors for the misuse of antibiotics
- Client’s negative attitude toward health institutions and around drugs is the most common factor for misuse of antibiotics

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of Nursing, Iraq and all experiments were carried out in accordance with approved guidelines.

**References**

Hemostasis in sickle cell disease

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³Assistant. Prof., Physiology department / Al- Zhraa medical college / University of Basrah, Iraq

Abstract
Sickle cell disease results in a significant morbidity and mortality related to intra-vascular thrombosis. This study is an attempt to improve our understanding the role of hypercoagulability in the pathogenesis of sickle cell disease. A case group of 20 asymptomatic sickle cell adult patients in a steady-state were compared with a control group of 20 normal adult people, both groups aged (18-50) year and from both sex, to evaluate the process of hemostasis. An investigation has been done for both groups including estimation of hemoglobin, platelets count, bleeding time and clotting time. There is a highly significant increase in the number of platelets with (P<0.01) together with a highly significant decrease in bleeding time with (P<0.01) for a case group in comparison with a control group. There is no significant difference of clotting time between both groups. That may suggest that the platelets aggregation formation activity is significantly increase in patients with sickle cell disease in a steady-state.

Keyword: Sickle cell disease, Platelets, Bleeding time

Introduction
Sickle cell disease (SCD) is an inherited lifelong disorder of genetic abnormality¹. Characterized by sickle (crescent)-shaped red blood cells and chronic anemia caused by excessive destruction of the abnormal red blood cells and multi-organ morbidity.² There is increasing evidence that SCD are usually associated with a hypercaogulable state and the risk of thrombotic complications appears to be higher in SCD patient.³

Hemostasis is a process of prevention the blood loss after vascular injury achieved by formation of clot,⁴ which is a mass formed from platelets and various blood proteins(clotting factors) within a blood vessels to stop bleeding.⁵

Platelets are tiny cell fragments that circulate in the blood stream and they are the first cells to react to blood vessels injury and seal off the wound, preventing more blood from escaping⁶.

The present study was designed to evaluate the platelets plug formation activity by measuring the bleeding time (BT) which is a standard test and basic assessment of how the blood platelets work to form a plug and stop bleeding, normal value is (2-7mints) for unisex and for all age groups.⁷ Also we measured the clotting time(CT),which is the time required for a sample of blood to coagulate in vitro, the normal value is (8-15mints) for unisex and for all age group.⁸ By this test we can evaluate the ability of different clotting factors work to form a clot normally.

Materials and Method
Case group of a total 20 steady-state SCD patients from both sex , aged between (18-50) years old, recruited from Al-Sadder teaching hospital outpatient medical clinic, documented as homozygous for HbS using hemoglobin electrophoresis. Compared with 20 healthy control group from both sex and have the same age. Investigations has been done for both groups including hemoglobin concentration, platelet count, bleeding time and clotting time.

Bleeding time procedure
Pricking a sterilized finger about 4mm depth, at the same time press the stop watch, after 30 sec. we remove the blood ooze from the finger by a filter paper every 30 sec. until the miner bleeding disappear⁹.

Bleeding time is consider as the number of blood spots + 2

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Clotting time procedure

Pricking a sterilized finger about 4mm depth, filling a capillary tube with blood, at the same time press the stop watch and start to break down the capillary tube every 30 sec. looking for the formation of fibrin thread⁹.

Results and Discussion

The study was in a completely randomized method and the results performed with SPSS statistical software version 20 ANOVA analysis of variation.

Probability value of (P<0.05) considered to be statistically significant.

Table 1: Visualize the mean ± SD of hemoglobin concentration and platelets count

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Case Mean ± SD</th>
<th>Control Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin Concentration</td>
<td>9.96 ± 1.216</td>
<td>11.54 ± 2.84</td>
</tr>
<tr>
<td>Platelets Count</td>
<td>402.37 ± 44.08</td>
<td>216.00 ± 5.03</td>
</tr>
</tbody>
</table>

*Statistically highly significant conceder as (P<0.01)

The comparison between SCD adult patients with normal people shows highly significant increase in platelets count with highly significant decrease in bleeding time and hemoglobin concentration (P<0.01), as visualized in table(1) and table( 2).

Table 2: Visualize the mean ± SD of Bleeding time & Clotting time

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Case Mean ± SD</th>
<th>Control Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding Time</td>
<td>1.82 ± 0.96</td>
<td>3.40± 1.48</td>
</tr>
<tr>
<td>Clotting Time</td>
<td>2.95 ± 1.82</td>
<td>3.38 ± 1.50</td>
</tr>
</tbody>
</table>

*Statistically highly significant decrease of bleeding time (P<0.01)

no significant differences for clotting time.

Sickle cell disease refers to a group of genetic disorders defined by the presence of sickle hemoglobin(HbS) that passed from parents to the children.¹⁰ This study is an attempt to improve our understanding the role of hypercoagulability in the pathogenesis of SCD.

The raised platelets count has previously been reported.¹¹ Platelets stimulation and chronic activation of coagulation is commonly observed in patient with SCD at steady-state compared to healthy control subjects with normal hemoglobin.¹¹ This is evidenced by increased plasma levels of in vivo markers of fibrin generation and thrombin, including fibrinopeptide A,D -dimers and plasmin-antiplasmin complex.¹² One of the most important factor that contribute to platelets activation in SCD, is the abundance of free hemoglobin in the circulation, which is responsible for the alteration of platelets functions by limiting the bioavailability of nitic oxide(NO).¹³ It has been reported that NO play very important role to inhibit platelets aggregations and adhesion to subendothelium matrices/endothelium through the cyclic guanosine monophosphate(GMP) pathway.¹⁴ Furthermore, circulating HbS can potentiate the platelets activation and promote the platelet-thromus formation on subendothelium matrix, also it can bound to glucoprotein (GPIba) and activated the intracellular proteins of Lyn_ERK pathway in the platelets.¹⁵ Increased plasma level of markers of platelets in SCD patients are capable of stimulating endothelial cell activation as demonstrated by the induction of the increased expression of endothelial inflammatory and adhesive proteins.¹⁶ Furthermore, hyper activity of
platelets also found to be more vigorous in SCD painful crises that may suggest a possible therapeutic role of drugs to inhibit the platelets functions.17

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Physiology department / college of medicine/ University of Basrah, Iraq and all experiments were carried out in accordance with approved guidelines.

References


Assessment of Primary Schools Teachers’ Knowledge and Attitudes toward Communicable Diseases Prevention and Control at Primary Schools

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Abstract

Background: Communicable diseases are illnesses that transmitted from a person, animal, or inanimate source to another person either directly or indirectly. Illnesses caused by communicable diseases are common among school students because of overcrowding.

Objectives: The purpose of the study is to assess the knowledge and attitudes of primary school teachers toward communicable diseases prevention and control and to find out the relationship between the primary school teachers’ knowledge and attitudes with their personal and socio-demographic characteristics.

Methodology: A descriptive cross-sectional study design using a simple random sample of (N=400) teachers working in primary schools through the period from October 2018 to June 2019. A questionnaire was created for this purpose. Its composed of (4) parts. The overall items included in the questionnaire are (55) items. A pilot study conducted to determine the reliability of the questionnaire, while the validity is obtained by distribution of the questionnaire through a panel of (15) experts. Statistical package for social sciences (SPSS) version (25) used for the analysis of the data. Appropriate statistical tests were used accordingly.

Statistical analysis used: The data are coded and tested by the application (SPSS) through the descriptive and inferential analysis.

Results: The study findings indicate that most of teachers (65.8%) had good knowledge toward communicable diseases prevention and control, also the highest percentage of teachers had agreed attitudes toward communicable diseases.

Conclusions and recommendations: All teachers working with children may be at risk for getting communicable diseases, especially those with less years of experiences. Therefore, it is necessary to increase their awareness toward communicable diseases prevention and control through collaboration with ministry of health and environment and through education programs about communicable diseases and ways of prevention.

Key words: assessment, teacher, knowledge, attitude, communicable diseases, prevention, control, primary school.

Introduction

A communicable disease is a disease caused by infection (invasion of the body) with specific germs such as viruses, bacteria, funguses, and parasites 1. Communicable diseases are still the major killers in the world, with tuberculosis, malaria and HIV taking a huge annual toll (approximately 4–5 million deaths). They are found particularly in conditions that encourage transmission, such as overcrowding or poor hygiene, so are more common in developing countries 2. Transmission of a communicable disease may occur through one or more of diverse pathways including physical contact with infected individuals.
These agents may be transmitted through liquids, foods, body fluids, contaminated objects, airborne inhalation. Illnesses caused by communicable diseases are common among school children and other childcare settings. Socioeconomic factors can increase the risk of outbreaks among school children in these settings. Health education can improve school population knowledge on communicable diseases and promote the development of appropriate behaviors toward communicable diseases. Health education should be provided for school population to validate positive health-promoting beliefs, intentions, and behaviors. Teachers are in a very powerful position. Their behavior, as a model and their opinions as to what constitutes good health, impact very directly on the concepts of health adopted by their school students. They are concerned in promoting some aspects of health, such as improving the self-esteem of their learners, teaching acceptable ways of relating to others and disease prevention.

School children are the greatest investment of any community and the main basis for its development. They are the biggest promise for the future. Communicable disease can be a major cause of illness among school children and can affect a child’s schooling by causing absenteeism.

Lack of knowledge about prevention and control of diseases lead to high risk of disease spreading causes increasing mortality and morbidity among school children.

### Methodology

The study aims to assess the knowledge and attitudes of primary school teachers regarding communicable diseases prevention and control.

**Study Design:** A descriptive cross-sectional study that conducted during the period of 30/7/2018 to 29/7/2019.

**Setting of the Study:** The study was conducted at the primary schools of Al-Hilla city which include (Hilla city center, Abu-Ghariq district and AL-Kefal district)

**Study Sample:** All primary school teachers (n=400) were included from (27) governmental primary schools who works at Al-Hilla city primary schools.

**Study instrument:** The information of data was collected through a questionnaire used to assess the knowledge and attitudes of primary school teachers toward communicable diseases prevention and control.

**Data Analysis:** The data were coded and tested by the “statistical package of the social sciences (SPSS) (Version 25)”. A descriptive analysis includes frequencies and percentage; inferential data analysis included Chi square test.

### Results

**Table (1): Relationship between the Overall Teachers’ Knowledge and their Socio-demographic and Personal Characteristics**

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<tr>
<th>Demographic data</th>
<th>Rating</th>
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### Table (1): Relationship between the Overall Teachers’ Knowledge and their Socio-demographic and Personal Characteristics

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“Chi-square observer, Chi-square critical, Df= Degree of freedom, P-value= Probability value, S= significant, NS= non significant, HS= high significant”

This table explains there was insignificantly association between primary schools teachers knowledge and personal characteristics at _p-value <0.05_.

Table (2): Relationship between the Teachers’ Attitudes about Prevention and Control and their Socio-demographic and Personal Characteristics

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Cont... Table (2): Relationship between the Teachers' Attitudes about Prevention and Control and their Socio-demographic and Personal Characteristics

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<th>Duration of the service</th>
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" Chi-square observer, Chi-square critical, Df= Degree of freedom, P-value= Probability value, S= significant, NS= non significant, HS= high significant"

This table shows there was insignificantly association between teachers attitudes toward prevention and control of communicable diseases and their socio-demographic and personal characteristics at p-value >0.05.

Discussion

Socio-demographic and Personal Characteristics of Primary School Teachers:

The results in the presented study indicated that (40%) of the teachers were within the second age group (33-43) years old and (32.25%) of them were between (44-54) years old, this results agreed with a study that was carried out in Iraq, the results of this study showed that two thirds of the sample were within (36-44) years old. Regarding gender, results indicated that (80.75%) of the sample under study were females and (19.25%) were males. The findings were in accordance with study which was reported that (78%) of their studied sample were female (Table 1). Also, the results of the present study revealed that the highest percentage of teachers (90%) were living in urban areas. The result disagreed with study conducted who revealed that half of the participants were living in urban areas. Furthermore, the results of the present study found that (24%) of primary school teachers were within (16-20) years of experience. This result agreed with a study revealed that (35.8%) of their studied teachers had (16-more) years of experiences. Concerning teachers education, the study revealed that more than half of them (55.3%) were accounted to be institute graduates. The result agreed with a study which revealed that more than half of their participants were institute graduates. This finding was not unexpected because in Iraq, colleges were not available in all Iraqi provinces prepare primary school teachers.

Relationship Between Teachers’ Knowledge and Their Socio-demographic and Personal Characteristics

(Table 2) indicated that there was no significant relationship between teachers’ general knowledge and
their age group in the study in which both young and old teachers had the same knowledge about communicable diseases. A study emphasized that there was non-significant relationship between teachers’ knowledge and their age groups. The study indicated that there is non-significant relationship between teachers’ knowledge and their gender characteristics in the study. A study found in her study there was non-significant relationship between variables in terms of the gender as they don’t contribute of developing teachers’ knowledge.

With concern to the data study of the insignificant relationship between teachers’ general knowledge and their marital status. The researcher found in that there was non-significant relationship in knowledge (Table 2). This result was inconsistent with a study found that there was non-significant relationship between teachers’ knowledge and marital status.

The study showed that there is insignificant relationship between teachers’ general knowledge and residency. Another study stated in that there was a huge connection between educators’ information and their living area territory. There is no significant relationship between teachers’ general knowledge and their economic status. The result of the study may be due sharing the same knowledge from the health coordinators.

Concerning the relationship between teachers’ general knowledge and their duration of services. The current study showed that there is insignificant relationship. Another study mentioned that if the teacher has five years of experience and more, they will have high level of knowledge about CDs.

Regarding the relationship between teachers’ general knowledge and their educational level. The current study showed that there is insignificant relationship.

Relationship between Teachers’ Attitudes and Their Socio-demographic and Personal Characteristics

The study showed that there is no significant relationship between teachers’ attitudes and their age groups, also study indicated that there is non-significant relationship between teachers’ attitudes and their gender characteristics. With concern to the study data of the significant relationship between teachers’ attitudes and their marital status. As well as study showed that there is insignificant relationship between teachers’ attitudes and residency. There is no significant relationship between teachers’ attitudes and their economic status. Concerning the insignificant relationship between teachers’ attitudes and their duration of services as shown in (Table 3). The study is inconsistent with a study which revealed that activity of teachers try to do it in their classes, the major of them were about personal hygiene, instructions to prevent buying from seller rover and there are less focusing on sexual transmission diseases.

Conclusions

The knowledge of primary school teachers about communicable diseases prevention and control in terms of information were moderately knowledgeable, while teachers knowledge about general information on communicable diseases prevention and control were having good knowledge. Attitudes of primary school teachers upon communicable diseases prevention and control were agreed attitudes.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Nursing and all experiments were carried out in accordance with approved guidelines.

References


Developing the Rear Circular Motion According To Auxiliary Device In The Ground Gymnastics

Wahab Abdul Wahhab Abdul Wahid1, Imad Kadem Yasser2

1M.Sc. Student, Directorate of Education al mathnaa, 2Assist. Prof. Physical Education and Sports Sciences / Thi- Qar University,

Abstract
Tests are among the priorities of science that reach us to scientific facts, including the processes of manufacturing devices and tools that are of great importance in the learning process, and in the sport of gymnastics, which has a particular character of the age group associated with the element of fear and anxiety as well as through the difficulty of the skills to be learned. Tools are among the most important factors that contribute to the speed of mastering learning skill while raising the side of anxiety and fear.

The researcher has reached through the conclusions that the designed device is important in the development of the skill to be learned

Keywords: development, circular motion of your gymnastics, by a factory device.

Introduction

The learning process is important in preparing the players from the early stages of childhood and creating the right conditions for them to acquire mental, physical and psychological experiences through different educational and training curricula, as the educational curricula contribute to some extent in the process of organizing and arranging exercises according to the scientific bases studied and the standards followed for Raise the level of achievement in various sports, including gymnastics.

Gymnastic sport is one of the basic sports of the Olympic Games and the tracker of this sport is noted the development of skills varied in terms of difficulty and technical performance on various devices, especially when they master the correct technical foundations through educational and training curricula, this sport is a long self in training and need to start early ages to build A fundamental basis for the learning process because it is a sport with a direct impact that affects the development of their mental, physical, skill and physiological abilities.

The interest of the developed countries in this kind of sport (gymnastics) has two considerations: because it builds the human being properly and soundly, and the second because it has an ample share of decorations equal to twice the medals in group games and other individual games, especially in the Olympic and regional tournaments as a result of the inclusion of multiple devices, and the amount of decorations The country has won 28 gold, silver and bronze medals.

As gymnastics in the world is developing rapidly, we need to keep up This development so as to keep pace with international in this area.

This will only be achieved through scientific research and the use of innovative scientific methods Learning, training and selecting the appropriate methods for the type of performance required in order to raise the level of the player Physically and physically.

Search problem:

Through the follow-up of the researcher for a number of competitions at the level of the country, especially the races of the age group under ten years and through personal interviews of a number of country coaches regarding the age group under (10) years found that it depends on personal experience in the process of learning motor skills in addition to that some trainers Engage the novice player in competitions before the process of mastering learning skills in this game, where the process of mastering skills in the game of gymnastics significantly contribute to determine the level of technical performance of the player and in the case of lack of skill will affect the outcome of the player and
the final team Lack of educational curricula based on the scientific basis to the lack of development in the level as well as to the lack of the player’s ability to develop these skills in the future to difficulty with higher skills.

For this purpose the researcher wanted this study, which investigates the problems of the group of reasons that address the real problem and put it in the hands of workers and trainers to work in the light of the results derived from the research.

Research Objectives

1 - Design auxiliary device for the development of circular motion in ground gymnastics.

2 - Identify the performance time of the circular movement of the members of the research sample.

3 - Recognize the differences in the research sample.

Research hypotheses:

1 - The variation of the period of time to perform circular motion among the members of the research sample.

2 - There are statistically significant differences between the members of the research sample

Research Methodology and Field Procedures:

Methodology and Research Sample

The experimental method was used in a one-group approach to suit the nature of the research problem to be solved. “The experimental method is one of the most efficient means of obtaining reliable knowledge that can be used to solve problems.” Recently to the Specialist Center in Muthanna. The research community consisted of (16) players. The device was applied to the community as a sample of the research experience and the percentage of the sample was (100%). Components of the device: The device consists of a set of parts that serve the performance of this skill.

* - Aluminum rail (2) length of 5 meters
* - Cylinder to move the arm in a circular
* - Iron wheels number (4)
* - Aluminum box length (30 cm and width of 5 cm).
* - Pods
* - Bluetooth device.

* - Circular arm of iron in the form of ()
* - Remote control panel
* - Battery (12) volts.

Machine specifications:

The device works by remote control through synchronization with the movement of the player and the amount of differences in terms of speed and the number of steps in the performance of this skill so that the performance of the jump stage is synchronized with the moment of jumping and controlled by the coach through direct viewing where the work of the inner cylinder in a circular tied to the arm that rotates with the movement of the cylinder and whose task is to lift the center of gravity for the player through a well-installed and tightly incubator, which is a wave towards the most important area to lift the center of gravity is represented by the hip at the end of the thighs from the bottom. This process is carried out through the fixed position of the performance and when Repeat this movement The device is also fixed in the same place of the player and when the repetition of continuous movement of the device works the same mechanism, but the coach is moving the device that works through Bluetooth in the horizontal direction that helps him in the movement is the presence of wheels that run over the aluminum rail and these duplicates The horizontal of the movement is a distance of (5) m length of two rails of aluminum each end at the end of the key to shorten the length of this rail to be easy to carry and transport and is used in more than one place, as shown in the following figure.
Application of the machine:

The goal is to teach players the skill required to learn the movements of the carpet movements of the circular jump in the game of gymnastics, which are basic skills that will be the basis for many other skills.

The researcher started the application on Sunday 6/3/2020 until 6/5/2020 and was carried out after giving the two modules to the research sample and then conduct the pre-tests where for (8) (Consecutive weeks) (3) units of instruction per week, including (24) units and the time of teaching unit (66) minutes in the main section as a special part to learn motor skills were taken into account the graduation in the size of the training where the time of exercise of the players (20) minutes of the device In the first and second week rest periods were 1 ring Ge included an side of my 2 minutes for each device either in the third and fourth week was lifted volume training by reducing the rest periods to half of 30 seconds with the survival of the theoretical side 2 minutes either on the fifth, sixth, seventh and eighth week.

Skill Time Measurement

Video was conducted to measure the skill time of the research sample and on the hall of the Muthanna Sports Club where each player performs two attempts for the skill of the hands jump back and small rear circle.

2.6 Statistical means:

\[ z = \frac{M - \bar{X}}{\frac{SD}{\sqrt{N}}} \]

3-4. View, analyze and discuss the search results:

Table (3): It shows the mean, standard deviation, calculated (T) values, spreadsheet, and type of statistical significance of the results of the pre and post circular motion of the sample

<table>
<thead>
<tr>
<th>Statistics Variables</th>
<th>Measure</th>
<th>Posttest 1</th>
<th>Pretest</th>
<th>(T)</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>X-</td>
<td>S d</td>
<td>X-</td>
<td></td>
</tr>
<tr>
<td>Circular motion of</td>
<td>Tame's</td>
<td>0.989</td>
<td>0.799</td>
<td>0.323</td>
<td>0.212</td>
</tr>
<tr>
<td>the back hands jump</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The reason for the development in the application is due to the auxiliary device in learning this movement as well as the process of transfer of the impact of learning between similar skills, whether in the exciting or in response to a skill, which has helped to learn the new skill. Learning between the parts of the skill in terms of perception and physical attributes and the use of repetitive attempts has a positive impact in increasing learning and development, since repetition forms of mathematical movements is to stabilize the field and dynamics of its progress, and in general be a clear and meaningful direction and to calculate the ability and leadership His hips.

The researcher believes that the application on the device has in addition to the motor performance in addition to the displacement of the factor of fear and anxiety and psychological “This occurs the beginnings of learning in early childhood because the child wants to learn, and this makes learning at this age stage feasible, and the researcher also believes that a good relationship Between the components of the load in terms of experimental size and rest periods have effectively contributed to the process of organizing a timely time so that the players move from one repetition to another in an organizational manner so that players have an understanding of the time of each exercise in learning a skill.

The researcher also states that the gradient in the difficulty of performance and by making the skill run smoothly by the auxiliary device to perform this skill on a straight line and for the purpose of expanding the base of perception of the player as the extension of the joints of the body with one line and make the center of gravity of the body perpendicular to the base makes the skill more consistent And aesthetic in performance, “the coordination and organization between tensile and relaxation in muscle contractions gives the flow of movement”.

Frafel says that “the basis of acceptance of information is subjective and that its integration serves only subjective information and when expanded, is sufficient to arrange the objective sense of motor measurement in other words, the greater the completion of objective information to the athlete and obtained whenever he was able to integrate the technique early”.

Conclusions and Recommendations

Conclusions:

1. The auxiliary apparatus has achieved its purposes and objectives for which it was established by teaching the performance of the skill to be learned properly.

2. The device contributed to the process of stimulation and response led to the transfer of learning between parts of the skill.

3. The use of instructional steps in the skill has a positive effect in improving Level of skill learning and technical performance.

Recommendations:

1. Attention to the stages of age below (10) years as it is essential in the construction of most different sports.

2. The need to use the gradient in teaching skills from easy to difficult of similar skills in the motivator and response for the purpose of benefiting from the transfer of learning from one skill to another skill.

3. Adoption of the device as an aid in educational institutions and training centers for the category of buds since the curriculum has achieved good results in teaching basic skills.

4. Conduct research and studies similar to the basic skills on the rest of the organs in gymnastics Professional.

Conclusion

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Physical Education and Sports Sciences and all experiments were carried out in accordance with approved guidelines.

References

The Effect of Different Cement Space on the Fracture Strength of Two Different Types Zirconium CAD/CAM Crowns

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Abstract

Objectives: Evaluate and compare the effect of three different cement space (50um, 70um and 90um) on the fracture strength of (vita zirconia crowns and Ips e-max Zir/cad zirconia crowns).

Materials and Methods: An ideal prepared plastic dentoform model of a left maxillary first molar with deep chamfer finish line design was used as a pattern for fabrication master metal die. The model was scanned and the master metal die was milled by CAD/CAM system. Sixty crowns made from two different types of zirconium were fabricated on a metal die and were divided into two major groups (A and B)( 30 crowns for each group) according to the two different types of zirconium being used: Group A: fabricated with vita zirconium crowns ; Group B: fabricated with IPS e-max Zir/CAD crowns. Each group further subdivided into three subgroups (10 crowns constructed with 50 um cement space crowns ), ( 10 crowns constructed with 70 um cement space crowns ) and (10 crowns constructed with 90um cement space crowns).

Results: The results of this study presented that the highest mean value of fracture strength is recorded via Group A1 (3.767± 0.242), followed by GroupA2 (3.266 ± 0.116), Group A3 (2.883 ± 0.085), Group B1 (2.505± 0.155), Group B2 (2 ± 0.141) and Group B3(1.508 ± 0.151) respectively. The data were statistically analyzed using one-way ANOVA and HSD tests which revealed highly significant differences (p<0.01) among groups. independent sample t-test result revealed a statistically highly significant difference between subgroups. Conclusion: Vita zirconium crowns is more resistance to fracture than IPS e-max Zir/CAD. (50um cement space) have higher fracture strength than the others.

Keywords: Cement space, Zirconia, CAD/CAM, Fracture strength.

Introduction

Cement space can be define is the vertical distance between the surface of the die and the inner surface of the restoration ¹. Advantage of using the die spacers permit increased space for the cement between the internal surface of the casting and the tooth surface, decreasing stress areas formed during cementation and thus resulting in better fit to the final restoration ². With the CAD/CAM system digital die spacer is very consistent in thickness regardless to the shape of the die (3, 4).

The overview of the technology of new CAD/CAM milling and new zirconia materials are ready possibilities for manufacture the monolithic zirconia crown ⁵, ⁶. The monolithic crown zirconia is the restoration of a full-contour crown zirconia designed without the addition of porcelain, which was industrialized to overcome the main complications problems of zirconia-based restoration is the veneer cracks ⁷, ⁸.

Zirconia has attracted maximum attention because it has the highest flexural strength and fracture toughness of all the existing ceramics. Natural phase transformation occurs internally in zirconia when an external stress is applied, which prevents crack propagation and tightens the crack tip, resulting in increase in the flexural strength and fracture toughness ¹⁰, ¹¹.

The Fracture strength of all-ceramic restorations is strongly dependent on the support materials. In addition,
cement type, cement thickness and preparation design can be influential factors. The film thickness of the cement affects directly the long-term clinical success. While determining film thickness of the cement, rate, mixing technique and heat are as much important as the clinician’s experience of the material. As a result, in real clinical situations, the actual cement thickness depends on the material used and the experience of the clinician.

**Materials and Method**

**Standardize die**

An ideal prepared plastic maxillary left first molar tooth (Nissin Dental Products, Kyoto Japan) with deep chamfer finishing line of (2 mm) occlusal reduction and axial reduction of (1.5 mm) was used to construction the master metal die.

Several CAD/CAM studies have used master metal dies (14,15,16). Then a metal die was fabricated by using CAD / CAM system to simulate the shape of ideal prepared plastic tooth to receive the zirconium crowns (17, 18).

The plastic prepared die was scanned using white light scanner (DOF, full HD, 2 M pixel , Korea) with stable scan stage method, then the digital model of the die sent to the milling machine (VHF S1, K5 impression machine, Germany) which was loaded with cobalt chromium disc 14mm ( Interdent, Travagliato (BS) Italy) to start dry milling process.

The metal base was flat and cubic in shape with length , width of (20 mm) and height of about (15mm) fabricated by using Computer Numerically controlled machine (CNC) figure (1).

**Sampling grouping**

Sixty crowns were introduced in this study which is divided into two groups according to the two types of zirconium and three different cement space as follows:

**Group A:** 30 crowns were constructed from (VITA Zahnfabrik Zirconia crowns).

**Group B:** 30 crowns were constructed from (IPS e-max Zir CAD Zirconia crowns).

Each group then subdivided into three subgroups of (10 crowns constructed with 50 um cement space crowns ), ( 10 crowns constructed with 70 um cement space crowns ) and (10 crowns constructed with 90um cement space crowns).

**Fabrication of crowns**

All manufacturing procedures containing model scanning, milling and sintering protocols. All procedures have been carried out in accordance with the manufacturer’s instructions for the zirconia and the CAD-CAM system used.

Exocad software (GmbH, Germany) were used to design the crown , the same design is programmed for both types of zirconium CAD/CAM crowns.

In this study three different cement gap thickness of 50 um (0.05mm), 70 um (0.07mm) and 90 um (0.09 mm), and an adhesive space of 1000um (1mm) from the finish line is used, Proximal contact :0 , Minimum occlusal thickness: 2000 um (2mm), Minimum circular thickness: 1500um (1.5mm), and 1000um (1mm) Margin thickness.
fracture strength testing procedure

Single load to failure test was used in the current study to measure the fracture strength of the crowns fabricated with the (vita zirconia crowns and Ips e-max Zir/cad crowns).

A (1 mm) thick piece of rubber was placed between the crown and the occluding rod to prevent peak load pressure on the crown surface and distribute the applied force over a larger area, i.e., homogenous stress distribution.\(^{(20,\ 21)}\) figure (2).

All samples were loaded until fracture and the maximum breaking load of each sample was recorded automatically in kilo Newton (KN) through a computer linked to the loading machine.

The mode of the fracture in this study divided into three types as follow:

Type one: Partial fracture of crowns from margin towards occlusal surface

Type two: Complete fracture of crowns through midline (half of crown displaced or lost (catastrophic failure))

Type three: Chipping at the finishing line.

Statistical Analyses

The data was statistically analyzed using SPSS (statistical package for social science) version 24. One-way ANOVA and Tukey HSD tests for comparing fracture strength among different cement spaces in each group. Independent samples t-test to test any statistically significant difference in the fracture strength between the two groups for each cement space.

Result

Table (1): Descriptive statistics of fracture strength (KN) with different groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Subgroups</th>
<th>N</th>
<th>Mean</th>
<th>S.D.</th>
<th>Min.</th>
<th>Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>VITA Zirconia</td>
<td>A1</td>
<td>10</td>
<td>3.767</td>
<td>0.242</td>
<td>3.52</td>
<td>4.12</td>
</tr>
<tr>
<td></td>
<td>A2</td>
<td>10</td>
<td>3.266</td>
<td>0.116</td>
<td>3.11</td>
<td>3.45</td>
</tr>
<tr>
<td></td>
<td>A3</td>
<td>10</td>
<td>2.883</td>
<td>0.085</td>
<td>2.77</td>
<td>3.01</td>
</tr>
<tr>
<td>IPS e-max Zir/CAD</td>
<td>B1</td>
<td>10</td>
<td>2.505</td>
<td>0.155</td>
<td>2.3</td>
<td>2.69</td>
</tr>
<tr>
<td></td>
<td>B2</td>
<td>10</td>
<td>2</td>
<td>0.141</td>
<td>1.78</td>
<td>2.15</td>
</tr>
<tr>
<td></td>
<td>B3</td>
<td>10</td>
<td>1.508</td>
<td>0.151</td>
<td>1.31</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Table (2): Comparison of fracture strength among different subgroups in each main group

<table>
<thead>
<tr>
<th>Groups</th>
<th>ANOVA</th>
<th>Sum of Squares</th>
<th>d.f.</th>
<th>Mean Square</th>
<th>F-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VITA Zirconia</td>
<td>Between Groups</td>
<td>3.930</td>
<td>2</td>
<td>1.965</td>
<td>74.268</td>
<td>0.000 (HS)</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>0.714</td>
<td>27</td>
<td>0.026</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>4.645</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IPS e-max Zir/CAD</td>
<td>Between Groups</td>
<td>4.970</td>
<td>2</td>
<td>2.485</td>
<td>111.905</td>
<td>0.000 (HS)</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>0.600</td>
<td>27</td>
<td>0.022</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>5.570</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3: Multiple comparisons between each two types of subgroups in each main group

<table>
<thead>
<tr>
<th>Groups</th>
<th>Subgroups</th>
<th>Mean Difference</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VITA Zirconia</td>
<td>A1 A2</td>
<td>0.501</td>
<td>0.000 (HS)</td>
</tr>
<tr>
<td></td>
<td>A3 A2</td>
<td>0.884</td>
<td>0.000 (HS)</td>
</tr>
<tr>
<td></td>
<td>A2 A3</td>
<td>0.383</td>
<td>0.000 (HS)</td>
</tr>
<tr>
<td>IPS e-max Zir/CAD</td>
<td>B1 B2</td>
<td>0.505</td>
<td>0.000 (HS)</td>
</tr>
<tr>
<td></td>
<td>B3 B2</td>
<td>0.997</td>
<td>0.000 (HS)</td>
</tr>
<tr>
<td></td>
<td>B2 B3</td>
<td>0.492</td>
<td>0.000 (HS)</td>
</tr>
</tbody>
</table>

Table 4: Descriptive statistics and subgroup difference regarding the fracture strength

<table>
<thead>
<tr>
<th>Subgroups</th>
<th>Descriptive statistics</th>
<th>Comparison (d.f.=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VITA Zirconia</td>
<td>IPS e-max Zir/CAD</td>
</tr>
<tr>
<td>Mean</td>
<td>S.D.</td>
<td>Mean</td>
</tr>
<tr>
<td>A1-B1</td>
<td>3.767 0.242</td>
<td>2.505 0.155</td>
</tr>
<tr>
<td>A2-B2</td>
<td>3.266 0.116</td>
<td>2.0 0.141</td>
</tr>
<tr>
<td>A3-B3</td>
<td>2.883 0.085</td>
<td>1.508 0.151</td>
</tr>
</tbody>
</table>

Discussion

The Computer aided design/ Computer aided manufacturing system are used in the manipulation of ceramics, such as zirconia or glass ceramics, as well as, this technology have the ability to produce an accurate fit and individual design, simple handling characteristics, and time consuming production processes; in addition to that, the CAD/CAM system demonstrated a variety from the options during scanning, design and during production of restoration. This system also permit the thickness of restoration and simulated die spacer (cement space) to be specified to the wanted thickness 11.

Zirconia fractures do not appear to be a common problem, although ceramics counting zirconia, this referred to as fragile materials 12, if monolithic zirconia crowns look to have adequate fracture resistance, the importance of cement should not be underestimated 5. It has been proven that the supporting materials, like cement and abutment material, will affect the fracture strength of all ceramic crowns 10.

Methodology

Using the Master die

In the current study, an ideal prepared plastic tooth #28 is used for the construction of a master metal die, 18.

The master die of Deep chamfer finishing line was selected because it improved the biomechanical performance of posterior single zirconia crown, which might be attributed to greater thickness and more rounded internal angles between the axial wall and gingival seat 16.

Then a metal die was fabricated by using CAD/ CAM to simulate the shape of an ideal prepared plastic tooth to receive the two types of zirconium crowns 17.
The advantages of using metal die include standardized preparation, better resistance to scratching, destruction or wear under repeated crown seating during fracture strength measuring procedure \( ^{14} \).

**Construction of metal base for the metal die.**

In the present study, the base used for the metal die is made from (cobalt chromium alloy) to allow proper position of the metal die during the test to prevent the fracture of the base \( ^{14} \).

The metal base was fabricated by using Computer Numerically controlled machine (CNC) \( ^{19} \).

**Using the single load.**

The single load to failure test was used to test the fracture strength of the crowns, because this test provides helpful data for comparing between the tested materials without the contribution of confounding variables brought from fatigue testing \( ^{21} \).

**Results**

In this study, Vita zirconium crowns fabricated with (50 um) cement space (Group A₁) showed a mean fracture strength of \( (3.767 \pm 0.242 \text{ KN}) \), while Vita zirconium crowns fabricated with (70 um) cement space (Group A₂) showed a mean fracture strength of \( (3.266 \pm 0.116 \text{ KN}) \) and Vita zirconium crowns fabricated with (90 um) cement space (Group A₃) showed a mean fracture strength of \( (2.883 \pm 0.085 \text{ KN}) \). IPS e-max Zir/CAD crowns fabricated with (50 um) cement space (Group B₁) showed a mean fracture strength of \( (2.505 \pm 0.155 \text{ KN}) \), while IPS e-max Zir/CAD crowns fabricated with (70 um) cement space (Group B₂) showed a mean fracture strength of \( (2.0 \pm 0.141 \text{ KN}) \) and IPS e-max Zir/CAD crowns fabricated with (90 um) cement space (Group B₃) showed a mean fracture strength of \( (1.508 \pm 0.151 \text{ KN}) \).

**Effect of cement space on the fracture strength.**

In this study, the result revealed that the decreased cement space causes increased fracture strength for all the groups (vita zirconia crowns (Group A) and Ips e-max Zir/CAD zirconia crowns (Group B)). This may be attributed to, that increase cement space measurement causes the crown thinner, because the strength required to fracture any crown is straight proportional with thickness of the material. Thus, thicker crowns will required more force to fracture than the thin crown. Therefor (90 um cement space) needed less force to break the crown compared with (50 um and 70 um cement space) . In addition, the increase cement space result in more space between the die and the crown, did not improve crown seating \( ^{0, 21} \).

These results came in agreement with \( ^{3} \) and \( ^{4} \) who found that the increased cement space causes decreased fracture strength.

**The effect of two types zirconium crowns on the fracture strength.**

The result of the current study about the fracture strength measurements of two types zirconium CAD/CAM crowns (Vita and Ips e-max Zir /CAD ) fabricated by three different cement space showed that the differences between the cement space was highly significant .

This coincide with \( ^{8} \) who conclude that the sintering time influenced the density and mechanical properties, they stated that the great sintering temperature and extended sintering period increased the grain size and thus result in a material with decreased mechanical properties.

In this study, restoration of the full-contour zirconia crowns with no ceramics overlay from all groups (A and B) is used to provide the crowns with adequate mechanical strength in order to resist occlusal forces while preserving biocompatible properties and excellent esthetics, Because of multilayered zirconium crown structures having loading capacity and distributions different from stress when compared to the monolithic restorations. Therefore, differences in fracture and mechanical behavior can be expected. In addition, the monolithic zirconia crowns, due to single composition of the anatomical blanks of zirconium material can be milled directly to full anatomical crown without the need for a ceramic veneer, and therefore not subject to an additional firing cycle compared to that of zirconia core materials \( ^{6} \).

**Conclusions**

Within the limitations of this study, the following conclusions can be derived:

1. The highest fracture strength mean value is recorded by crowns fabricated with (50um cement space) whereas the lowest mean value of fracture strength is recorded by fabricated crowns with
Differences were present in the fracture strength between two types of zirconium crowns; that show Vita zirconium crowns is more resistance to fracture than IPS e-max Zir/CAD.

The result of this study about the fracture strength measurements of two types zirconium CAD/CAM crowns (Vita and Ips e-max Zir /CAD ) fabricated by three different cement space showed that the differences between the cement space was highly significant.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Collage of Health and Medical Technology, Iraq and all experiments were carried out in accordance with approved guidelines.

References
20. Zesewitz TF, AW Knauber, FP Nothdurft, Fracture resistance of a selection of full-contour all-ceramic

Compliance of Hypertensive Patients with Medications at Marjan Teaching Hospital in Babylon Governorate

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2Lecturer, College of Nursing/University of Thi-Qar /Iraq

Abstract
Hypertension is a significant problem around the world. As indicated by World Health Organization. Objectives: The aims of this study was to assess the compliance of hypertensive adults with their treatment, and to assess their socio-demographic characteristics in relation to compliance. Methodology: The study was conducted at the medical outpatients’ clinic in a period from 1st November 2018 to 10th march 2019 Marjan Teaching Hospital. Collecting data from simple random sampling total of (50) hypertensive patients who are using antihypertensive treatment and attending the medical outpatients’ clinic at Marjan Teaching Hospital in Babylon Governorate. Results: The majority of the study participants were female who accounted for (64%) of the total participants while male constituted (36%). Most of the study participants (42%) were between ages 60 years old more. Most of the respondents (63%) were diagnosed more than five years ago. (44%) of the sample have poor level of compliance with medications, and (50%) had poor control of blood pressure. Conclusions: There is very low rate of medication compliance, and BP control with strong association between them, that reinforce the relation between them.

Keywords: Compliance, Hypertension patients, Medications at Marjan Teaching Hospital

Introduction
Hypertension is a significant problem around the world. As indicated by World Health Organization, hypertension influences 970 million individual and approximately 68 million adults have hypertension. Globally the overall propagation of hypertension in population between age 25 and more was about 40% in 2008. Hypertension is constitutes a predisposed factor for heart and vascular diseases which results precocious death. Hypertension is known as abnormally high systolic or diastolic blood pressure levels. It means continual systolic blood pressure (SBP) equal to or greater than 140 mmHg and/or continual diastolic blood pressure (DBP) equal to or greater than 90 mmHg. This increasing of (SBP) and (DBP) is well distinguished as an important risk factor for brain stroke coronary heart disease (CHD), (ESKD) and surprising dying.

According to the American Heart Association, (2013) there are several factors influences compliance medication regimen to hypertensive: knowledge of treatment, long period going treatment, level of education, unread and write, and bad association with health care services, attitudes toward medication and adverse effects, psycho social factors, economics status, getting to health care, absence of health insurance, disease without symptoms and inconvenience. Usually treatment non- adherence is linked with progress age, males, poorness, obesity, cigarette smoking, disease embattle, and lower social support.

Based on high prevalence and burden of HT, achieving best control rates of HT is top priority internationally, but control rates still low worldwide. Chow et al. 2013 in a multinational study indicate only 32.5% control rate of HT (Systolic Blood Pressure (SBP) <140 mmHg and Diastolic Blood pressure (DSP) <90 mmHg) and it was 20.7%, 33.6%, 38.5% in Africa, Middle East and North America and Europe respectively.

Compliance is a significant problem in HT and evidence shows that just half of patients who initiate drug therapy are adherent with treatment after 1 year in USA, and poor or noncompliance produce uncontrolled HT
that will lead to worsen condition and higher possibility for developing complications including ischemic heart disease, renal failure and cerebrovascular accidents and others \(^{(5,6)}\). A shortage of knowledge about the severity of the disease and the importance of compliance to the prescribed treatment, may constitute barriers to compliance behavior. Ensuring patients’ compliance with anti-hypertensive medications and lifestyle modifications to prevent complications of hypertension remains a major challenge to public.

Nurses, can play an essential role in facilitating patient adherence to the prescribed treatment regimen. As they responsible to help patients acquire knowledge, skills and change attitude necessary to maintain compliance, they plays an important role on helping the people learn to live with and control his hypertension, to encourage compliance with antihypertensive therapy \(^{7}\).

This study conducted at the medical consultation clinics of Marjan Teaching Hospital in Babylon Governorate. The aims of this study were to assess the compliance of hypertensive adults with their treatment, and to assess their socio-demographic characteristics in relation to compliance.

**Methodology**

A descriptive cross sectional study was conducted at the medical consultation clinics of Marjan Teaching Hospital for adult patients with hypertension that were on follow up as outpatients in clinic of the Hospital in Babylon. Oral consent was taken from each patient before to interview, after a brief explanation on the study and its objectives.

The study was conducted from 1\(^{st}\) November 2018 to 10\(^{th}\) March 2019 Marjan Teaching Hospital in Babylon Governorate.

The study population consisted of (50) adult hypertensive patients on treatment and attending outpatient clinics in the hospital. These patients are followed up in the hospital for regular treatment and checkups depending on high blood pressure control. A structured interview questionnaire that was developed by the researchers according to literature review. It divided into two parts. Part I: Demographic Information Sheet (14 questions). Part II: compliance with medication regimen (8 questions). All the items responses were noted on a 3-point Likert scale. The response options were: every never (1), frequently (2), always(3).

The validity of the questionnaire was confirmed by (10) experts. A self-administered structured questionnaire was used to collect the socio-demographic characteristics of the patient’s respondents, Factors that influence treatment compliance. Data collection from January to February 2019. The data were analysed through Statistical Analysis was done using Statistical Package for Social Sciences (SPSS V 21.0).

**Results**

![Figure 1: Reasons of poor compliance to medication](image)

![Figure 2: Compliance to medications according to the items responses among hypertensive patients](image)
Figure 3: Blood pressure control levels among patients with HT

Table 1. Association between compliance to medications and socio-demographic Characteristics

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Compliance to Medication</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Poor</td>
<td>Medium</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>8</td>
<td>16.0</td>
</tr>
<tr>
<td>Female</td>
<td>14</td>
<td>28.0</td>
</tr>
<tr>
<td>P-value</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td>Age (Years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 – 39</td>
<td>6</td>
<td>12.0</td>
</tr>
<tr>
<td>40 – 59</td>
<td>9</td>
<td>18.0</td>
</tr>
<tr>
<td>≥ 60</td>
<td>7</td>
<td>14.0</td>
</tr>
<tr>
<td>P-value</td>
<td>0.233</td>
<td></td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>20</td>
<td>40.0</td>
</tr>
<tr>
<td>Single</td>
<td>2</td>
<td>4.0</td>
</tr>
<tr>
<td>Others</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>P-value</td>
<td>0.110</td>
<td></td>
</tr>
<tr>
<td>Residence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>11</td>
<td>22.0</td>
</tr>
<tr>
<td>Rural</td>
<td>11</td>
<td>22.0</td>
</tr>
<tr>
<td>P-value</td>
<td>0.385</td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Government Employee</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Student</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Housewife/Jobless/retired</td>
<td>20</td>
<td>40.0</td>
</tr>
<tr>
<td>Free work</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>P-value</td>
<td>0.391</td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>unlearned</td>
<td>10</td>
<td>20.0</td>
</tr>
<tr>
<td>Primary school</td>
<td>3</td>
<td>6.0</td>
</tr>
<tr>
<td>Secondary school</td>
<td>8</td>
<td>16.0</td>
</tr>
<tr>
<td>Institute and more</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>P-value</td>
<td>0.144</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>2</td>
<td>4.0</td>
</tr>
<tr>
<td>Non smoker</td>
<td>20</td>
<td>40.0</td>
</tr>
<tr>
<td>P-value</td>
<td>0.064</td>
<td></td>
</tr>
<tr>
<td>Sleeping</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 8 hours</td>
<td>7</td>
<td>14.0</td>
</tr>
<tr>
<td>8 hours</td>
<td>15</td>
<td>30.0</td>
</tr>
<tr>
<td>More than 8 hours</td>
<td>0</td>
<td>0.0</td>
</tr>
</tbody>
</table>
The table revealed that the majority of the study participants were female who accounted for (64%) of the total participants while male constituted (36%). Regarding age (42%) were from the age group (60 years and more); (84%) of the patients were married, and (58%) lives in urban area. According occupation (86%) were housewives/ jobless/ retired, (52%) were unlearned (illiterate or read and write), and (90%) were non-smokers, while sleeping pattern (68%) were of them sleeping 8 hours. Regarding number of drugs (80%) were have one drug, (48%) were have not enough monthly income, and (48%) were have normal weight.

According reasons of poor medications the forgetfulness was the first cause of non-compliance to medication (46%), followed by negligence and carelessness of patients (30%), side effect (10%), asymptomatic nature of HT disease (8%), and cost of drug (6%), according to patients opinion was and as shown in Figure 1. And (44%) of the sample have poor level of compliance with medications Figure 2, (50%) had poor control of blood pressure, as shown in Figure 3.

**Discussion**

The present study was carried out to determine the
compliance of patients with hypertension with their treatment regime. The results of the study revealed that the majority of the study participants were women who accounted for (64%) of the total participants while male constituted (36%), the female were more compliant (14%) compared with male (6%). Most of the study participants (42%) were between ages 60 and above years old, the most participants who were (40-59) years of age had good level of treatment compliance compared to those with 60 and above years of age. (84%) of the patients were married, married group has highest percentage of acquiescence toward medication compliance. (52%) of the participants had unlearned. The majority of the participants were (58%) lived in urban. Majority of them (54%) were diagnosed between than (1-5) years.

These results are in conformity with the findings obtained from other studies, who state that high proportion of clients with whole medication compliance corresponded to the female (66.7%), The participated sample were age from (40 – 80) years ,approximately two-third of patients (68.12%) that ages over 60 years , and residual of them 24.63% age between 50 and 59 years (8,9).

This finding was coincided to a study conducted by other researchers stated that medication compliance rates were also significantly higher between ages (45-64) than younger or older age groups. The majority (63%) lived in urban areas while the rest (37%) lived in rural areas. Majority of them (63%) were diagnosed more than five years ago 10.

There are many factor affect the patients drugs treatment compliance, the main cause for poor compliance to medication as the patients indicates was forgetfulness to take medications (46%) (figure 1), they taking medications only when they develop symptoms such as headache and not take medication when they feel comfortably. Poor knowledge of the disease and ignorance of need for long-term treatment was reported as main cause by Busari et al 2010 11 , whereas self-awareness of disease remission was second cause of noncompliance in Dong et al 2013 12.

The results of present study show low rate (20%) of patients with good compliance with medication, whereas the majority of the patients (44%) had poor compliance to medication (figure 2). The low rate of compliance is less than that reported by different researchers that were (70%, 67.1%, 53.4%, 43.5%, 32.1%) respectively 13,14,15,16,17. While it was higher than the 13.24% compliance rate reported by Dosee et al 2009 18. Low BP control (50%) obvious in this study, with the fact of the direct association between better compliance to treatment with higher control rates of blood pressure make it a serious issue (figure 3). This differences in results from different societies could be related to variation in cultures, educational standards, beliefs about disease and treatment, as well health services development but still compliance to medications and control of BP is weak the worldwide.

**Conclusions**

1. There is no significant relationship between social demographic characteristics except gender, no. of drugs, and duration of diagnosis.
2. There is very low level of medication compliance, and low level of BP control with, and there is a strong association between them, that reinforce the relation between them.
3. The main reasons for noncompliance were forgetfulness, followed by negligence then side effect of medications.

**6. Recommendations**

1. Educate the patients about the use of their medication should be done diligently, primarily by the prescriber then health care professional.
2. Educate the patients to avoid high risk complication that threatening life result from poor treatment compliance.
3. Health education programs about hypertension should be campaigns state through the mass media as television and radio, social media, illustrated booklet, and pictures.
4. Health education programs for the healthcare professionals to arise their awareness, attitude and adaptation of the patient as to their own disease.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the College of Nursing and all experiments were carried out in accordance with approved guidelines.
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Immunization Status of Children Under 6 Years Old in Holly Karbala Governorate

Muhsan Sahib Salih Almusawi
Karbala Health Directorate, Iraq

Abstract
A cross-sectional study was carried out among 110 mothers of children to assess the immunization status of the children, they selected by using non probability sampling (convenience sampling). According to study findings, most of children were males (25.7%) and (95.5%) of them from urban resident and most of children have a good immunization status. The study concluded that performance of Iraqi national immunization program is effective. Keywords: Immunization Status, Children Under 6 Years Old.

Keywords: Immunization, Children, Karbala Governorate.

Introduction
Immunization has been described as the first line of defense against disease, and one of the most effective health advantages available to children. It is proven as one of the most cost effective health interventions worldwide, through which a number of childhood diseases have been prevented or eradicated. Immunization against diseases is one of the most important public health interventions with cost effective means to preventing childhood morbidity, mortality and disability. The term dropout refers to children who miss scheduled vaccinations for any reason, including health facility problems such as canceled sessions or vaccine stock outs. Immunization contributes significantly to the achievement of millennium development goal number 4 and is one of the eight elements of primary health care. Effective utilization of immunization services is associated with reduced infections in young children with immature immune system and improved child health outcome. The failure of immunization is result of postponing it until another time, child being ill and hence not brought to the center for immunization, un aware of the need of immunization, place of immunization being too far, no faith in immunization, un aware of the need to return for 2nd and 3rd dose, mother being too busy, fear of side reactions, and wrong ideas about immunization. Improvements in national immunization program performance are necessary to reach and sustain high vaccination coverage to increase protection from vaccine preventable diseases for all persons.

Methodology
Subjects: The study population included (110) mothers of children under 6 years selected by nonprobability sampling method (convenience sampling) in the medical health center of Holly Karbala Governorate. The researcher explain the study and the objective to the sample and take their oral consent to participate in the study, then collect the data about the subject.

Instrument: The researcher using a questionnaire consist of 2 parts: the 1st part contain the general information and the 2nd part contain the Iraqi immunization schedule which contain the vaccination from birth to 6 years. The data was analysis by using the descriptive statistical analysis (frequency and percent).
Results

Table (1) distribution of the study sample by their general information

<table>
<thead>
<tr>
<th>Variables</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>52</td>
<td>47.3</td>
</tr>
<tr>
<td>Male</td>
<td>58</td>
<td>52.7</td>
</tr>
<tr>
<td>Total</td>
<td>110</td>
<td>100</td>
</tr>
<tr>
<td>Age by years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>105</td>
<td>95.5</td>
</tr>
<tr>
<td>Rural</td>
<td>5</td>
<td>4.5</td>
</tr>
<tr>
<td>Total</td>
<td>110</td>
<td>100</td>
</tr>
</tbody>
</table>

No. = number, % = percentage

This table indicate that (52.7%) of children were males and (47.3%) were females, (95.5%) of them were urban resident and (4.5%) were rural.

Table (2) Immunization Status of Children Under 6 Years (n=110)

<table>
<thead>
<tr>
<th>Periods</th>
<th>Variables</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>f</td>
<td>%</td>
<td>f</td>
</tr>
<tr>
<td>1-7 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>BCG) Bacillus – Calmette – Guerin</td>
<td>106</td>
<td>96.4</td>
</tr>
<tr>
<td></td>
<td>Oral polio - zero dose</td>
<td>89</td>
<td>80.9</td>
</tr>
<tr>
<td></td>
<td>Hepatitis B ( Hep –B-)</td>
<td>87</td>
<td>79.1</td>
</tr>
<tr>
<td>2 months</td>
<td>Hexaxim vaccine - first dose</td>
<td>86</td>
<td>78.2</td>
</tr>
<tr>
<td></td>
<td>Oral polio - first dose</td>
<td>102</td>
<td>92.7</td>
</tr>
<tr>
<td></td>
<td>Rotavirus vaccine - first dose</td>
<td>18</td>
<td>16.4</td>
</tr>
<tr>
<td></td>
<td>Pneumococcal vaccine - first dose</td>
<td>61</td>
<td>55.5</td>
</tr>
<tr>
<td>4 months</td>
<td>Hexaxim vaccine - second dose</td>
<td>92</td>
<td>83.6</td>
</tr>
<tr>
<td></td>
<td>Oral polio – second dose</td>
<td>98</td>
<td>89.1</td>
</tr>
<tr>
<td></td>
<td>Rotavirus vaccine – second dose</td>
<td>18</td>
<td>16.4</td>
</tr>
<tr>
<td></td>
<td>Pneumococcal vaccine – second dose</td>
<td>53</td>
<td>48.2</td>
</tr>
<tr>
<td>6 months</td>
<td>Hexaxim vaccine - third dose</td>
<td>86</td>
<td>78.2</td>
</tr>
<tr>
<td></td>
<td>Oral polio – third dose</td>
<td>86</td>
<td>78.2</td>
</tr>
<tr>
<td></td>
<td>Pneumococcal vaccine – third dose</td>
<td>57</td>
<td>51.8</td>
</tr>
</tbody>
</table>
This table indicates that according to the immunization schedule of the children age 18 months, (96.4%) of children had receive the BCG vaccine, (80.9%) of them had receive oral polio- zero dose and (79.1%) of them receive hepatitis B viral at period (1-7) days. (78.2%) of them receive Hexaxim vaccine - first dose, (92.7%) of them receive Oral polio - first dose, (83.6%) not receive Rotavirus vaccine - first dose and (55.5%) receive Pneumococcal vaccine - first dose at period 2 month. (83.6%) of them receive Hexaxim vaccine - second dose, (89.1%) receive Oral polio – second dose, (83.6%) not receive Rotavirus vaccine – second dose and (51.8%) not receive Pneumococcal vaccine – second dose at period 4 months. (78.2%) receive Hexaxim vaccine - third dose, (78.2%) receive Oral polio – third dose, and (51.8%) receive Pneumococcal vaccine – third dose at period 6 month. (83.6%) receive Single measles vaccine and (72.7%) take Vitamin A – 100.000. IU at period 9 months. (76.4%) take (MMR) Mixed measles vaccine (measles, rubella, mumps) at period 15 months. (70.9%) take Second pentagon vaccine, (73.6%) take Polio vaccine - first active dose and (60%) take Vitamin A – 200.000. IU at period 18 months. (66.4%) not take The fourth quaternary vaccine, (75.5%) not take Oral polio vaccine - second active dose and (84.5%) not take MMR Mixed measles vaccine at period 4-6 years.

**Conclusion**

The study concluded that the children have a good immunization status through the Iraqi immunization schedule, the children were take most of vaccines and there was some of children dropout of the rotavirus vaccine at the 1st and 2nd dose that may be related to absence of vaccine vial in the health care centers or there was some factor like diseases or so far distance or environmental factor that make the families late to the vaccine date.

**Recommendation**

Using the mass media to encourage family about benefits of vaccine, give the vaccine to the families whom late the date of vaccination and provide the vaccines to the health care center and prevent deficiency.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Karbala Health Directorate, Iraq and all experiments were carried out in accordance with approved guidelines.

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Spectrophotometric Determination of Isopropamide Iodide based on Ion-pair Complex Formation with Thymol Blue

Wasan Ajam Hannoun1, Khalid Waleed S. Al-Janabi2

1M.Sc. Student, 2Assist. Prof., Chemistry Department, College of Education for Pure Sciences/ Ibn Al-Haitham, University of Baghdad, Baghdad, Iraq; Email: khalid.janabi@gmail.com

Abstract

A simple, accurate, affordable, and rapid spectrophotometric approach has been established for the quantification of the medicinal compound of Isopropamide iodide (ISO) in pharmaceutical preparations. An ion-pair complex which absorbs at 404nm has formed the reaction of the drug and the coloring reagent of Thymol blue (TB) at an acidic medium of pH=4. The calibration curve showed excellent linearity over a quantification range of (1-40 μg/ml) with an excellent coefficient of correlation (r = 0.9993). The examined limit of detection (LOD) was (0.3 μg/ml) for repeatability of (n= 3). The approach witness no interference of the commonly used excipients in such pharmaceutical formulation that contains Isopropamide iodide. Validation of the method was accomplished by calculating the relative standard deviation (RSD %) and found to be in the range of (1.1938-0.5954). While recovery percentage for (n=3) was (97.4 – 101.4%).

The analytical parameters of the method were optimized and were efficaciously applied to the Isopropamide iodide determination in pure form and in the mixture of a tablet form. The approach can be functional for determining other active pharmaceuticals.

Keywords: Isopropamide iodide, spectrophotometry, ion-pair complex, Thymol blue, pharmaceuticals.

Introduction

Isopropamide iodide (ISO), in IUPAC “(4-amino-4-oxo-3,3-diphenylbutyl)-methyl-di(propan-2-yl) azanium;iodide”, an off-white odorless bitter powder with a molar mass of 480.434 g/mol (1,2) as it shown in (Figure 1). Its an anticholinergic medication having long effect duration3. ISO is a wide spectrum pharmaceutical drug used in the treatment of several disease symptoms, not limited to; Assist in gastrointestinal (GI) spasm relief4, the remedy of peptic ulcer, thyroid disorder5, rhinitis redaction, some types of diarrhea, duodenitis spasm, chronic cholelithiasis, biliary dyskinesia, curing of gastritis, rest of spastic colon, disturbance of the urinary tract6 those correlated to the spasm of smooth muscle, and some other disorders7–10.

The Iraqi “State Company For Drugs Industry And Medical Appliances (SDI – Samarra)” produces tablets for the treatment of “irretable bowel syndrome” called Salabid which composed of ISO and trifluperazine hydrochloride11.

A literature survey revealed that ISO has been determined in the presence of other active drugs by derivative spectrometry12,13. Also, it has been extracted from physiological fluids via solid-phase extraction cartridges14 (SPE) followed by the chromatographic analysis of HPLC at 220 nm15,16. Likewise, platforms of electrochemical sensing were used for a simultaneous determination of ISO in a bulk pharmaceutical mixture17.

Fig. 1. The chemical structure of the pharmaceutical molecule of Isopropamide iodide15
A literature survey comes out with different approaches for finding the concentration of ISO in pharmaceutical mixtures. Many spectrophotometric approaches have been described for the quantification of ISO and measurement were accomplished within the ultraviolet (UV) region. A sensitive, rapid, accurate, precise and economical spectrophotometric method based on simultaneous equation method for the simultaneous estimation of isopropamide and trifluoperazine in combined tablet dosage form. Materials and Methods: The method is based on the simultaneous equation and first-order derivative method for analysis of both the drugs using methanol:water in the ratio of 7:3 (v/v). Simultaneous spectrophotometric estimation of ISO in the existence of trifluoperazine as a tranquilizer and its oxidative degradation was reported as well. Frequently, trifluoperazine comes in a dualistic mixture with ISO for their anti-vomiting and anti-convulsive influence. Similarly, derivative spectrometries were reported also since they have the privilege of simultaneous analysis of multi-component systems. Usually, as a subject to second derivative measurement within the UV region in a methanolic medium or in a slightly basic medium of 0.1 N sodium hydroxide followed an extraction step with chloroform. The procedure provides a linear quantification range for the determination of ISO within 20-80 µg/ml. However, complexation with methyl orange or sodium taurodeoxycholate as an ion-pair, or with iodine as a charge-transfer complex were common.

ISO can be formally determined ion-exchange liquid chromatography, or by direct titration with perchloric acid in an organic medium.

The proposed method was built on the reaction of Isopropamide iodide with Thymol blue in an acidic medium at 404 nm. The method proved to be effective for the determination of Isopropamide iodide in pure form and its pharmacological formulation.

Materials and Method

Instrumentation

- Spectrophotometric measurements were made by a UV-Visible Spectrophotometer (T80+ PG Instruments Ltd.).
- Analytical Electronic balance (Sartorius, with four decimals).
- The acidity of the prepared solutions and buffers were adjusted using a digital pH meter (pH/mV Bench Meter, Hanna Instruments) equipped with a combined glass pH electrode.

Material and Reagents

- ISO has been provided freely by “The State Drug Industries and Medical Appliances Company” (SDI), Samarrah, Iraq.
- Solvents and chemicals were of analytical grade.
- Thymol blue (TB), 0.1% (w/v) stock solution: a 0.1 g of the reagent TB was dissolved in 5 ml of methyl alcohol and then diluted with distilled water to 100 ml. While the standard working solutions of TB were prepared freshly by consequent dilutions.
- Hydrochloric acid (0.1M): A 0.83 ml of a concentrated HCl (Sp.gr. 1.18 g/ml, 37% w/w) was diluted into 25 ml of deionized distilled water then adjusted to a 100 ml.
- NaOH (0.1 M): the solution was prepared by dissolving 0.40 g of sodium hydroxide in 100 ml of distilled water.
- Preparation of copper sulfate solution was accomplished by dissolving a 0.25 g of CuSO₄ and 4.5 g of CH₃COONH₄ with the solution of 0.1M acetic acid in a 100ml volumetric flask. Adjustment of acidity was fulfilled using the dilute solutions of HCl and NaOH previously prepared.
- Preparation of ISO stock solution (100 µg/ml): 0.01g of ISO was dissolved in 100ml of distilled water.

Assay procedure of pure ISO

One milliliter of the ISO standard solution (20µg/ml) was moved into a set of 50 ml separatory funnel with a 0.5 ml of pH 4 buffer solution as well as a 0.5 ml of the reagent TB (.). A half milliliter of chloroform was added and the separatory funnel was shaken for one minute. The funnel was kept aside to allow a clear separation of the binary phase. Spectrometric measurement of the yellow organic phase was accomplished at a maximum wavelength of 404 nm against a blank. The calibration curve was built by graphing the absorbance intensity of the organic phase as a function to the medicine concentration.
Analysis of pharmaceutical formulation

Ten tablets of (Salabid tablets, a product of SDI) was ground and weighed accurately. Aliquot of 5mg of the fine powder was transferred quantitatively and dissolved into a 100ml of distilled water. The resulting solution was filtered to avert the presence of any suspended particles. Whereas, the standard working solutions were made by successive dilution.

Results and Discussion

In general, spectrometric procedures are well known as sensitive approaches for the determination of medicinal compositions. Spectrometric ion-pair complexation is commonly used in the determination of the concentration of various pharmaceutical composites.

A series of experimental investigations in buffer mediums have revealed the possibility of ISO compound to react with TB. The resulting yellow ion-pair derivative has been extracted from the aqueous medium by chloroform. The complex was formed in an acidic buffer medium and found to exhibit an absorption peak with a maximum at the wavelength of 404nm as its obvious in (Figure 2).

Fig. 2. The absorption spectrum of the ISO-TB yellow complex

Optimization of experimental variables

Univariable method

The experimental variables affecting the stability and the formation yield of the produced colored complex. They have been specified by the application of different sets of preliminary experiments. These variables include pH, reaction time, shaking time, reagent concentration, the order of addition, and type of the extraction solvent. Thus, the univariable approach was accomplished by modifying one variable at a time while keeping the other variables at constant values. Once the optimum value of the variable gets specified, then another parameter will be chosen for modification while maintaining all the others constant.

Effect of acidity

For the purpose of finding the optimum acidity range, ISO solutions were mixed separately with the coloring reagent of TB in mediums of different pH(s). The acidity of the reaction mediums was adjusted to a value between (2 - 5) via the addition of a few drops of either (0.1M) NaOH or (0.1M) HCl. Maximum absorbance was obtained at pH 4.0 (Figure 3). Therefore, an acidic medium with a pH of 4 was employed in all the subsequent preparations.

Fig. 3. The absorbance intensity for the formation of the ion-pair complex as a function of the pH of the reaction medium

Effect of reaction time

The optimization of the reaction time was accomplished by examining the development of the yellow color of the formed complex at ambient temperature (25 ± 2 °C). The reaction was instant as the colored product reached its maximum intensity with stable absorbance directly after the blending of ISO with the reagent of TB. The color intensity remains stable for the next 24hr (Table 1).
Tab. 1. The reaction time for the formation of the ion-pair complex as a function of absorbance intensity

<table>
<thead>
<tr>
<th>Time reaction (min)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.256</td>
</tr>
<tr>
<td>2</td>
<td>0.251</td>
</tr>
<tr>
<td>3</td>
<td>0.252</td>
</tr>
<tr>
<td>4</td>
<td>0.251</td>
</tr>
<tr>
<td>5</td>
<td>0.250</td>
</tr>
</tbody>
</table>

**Effect of reagent mole ratio**

Absorbances were measured when different volumes of the TB solution in the concentration of ( ) were added to 0.5ml of ISO standard solution (20μg/ml). An increment in TB concentration causes a reduction in the absorbance which may be due to the formation of new species (Figure 4).

**Optimization of the extraction shaking duration**

Optimization of the shaking time required to achieve a maximum extract recovery was studied over a time range of 1-5 minutes at ambient room temperature. No significant influence was observed for this parameter (Figure 5). However, a one minute found to be enough as an optimum shaking time for all the upcoming extractions.

**Effect of the extracting solvent**

Various organic solvents; Benzene, carbon tetrachloride, dichloromethane, cyclohexane, and chloroform, were inspected for their extractability ISO colored complex. Chloroform observed to have the most efficient extraction ability (Figure 6). Furthermore, experiments exhibited that a single extraction of a 5ml batch of chloroform was very satisfactory in terms of recovery.

**Influence of the order of addition**

The sequence of addition of the reactants is important and has been tested. The optimum order was by dissolving the ISO drug into the buffer then adding TB reagent to it. Starting with the ISO in the buffer gave a higher absorption (0.257 au) at the wavelength of 404nm than by starting with TB in the buffer (0.141 au).

**Stoichiometry of the complex**

The mole ratio of ISO to TB was established via Job’s method of continuous variation. Two standard solutions of ISO and TB both have the same concentration of were prepared. The reagent TB was added gradually to ISO buffer solution by a 0.1 ml portion so that the total volume of the mixture maintains to 1ml and the
absorption at the wavelength of 404nm is measured with each addition. The resulted graph has exhibited that the ion-pair complex is formed with a mole ratio of 1:1 to ISO:TB (Figure 7). As one positive protonated ISO molecule requires one anion of TB to form an ion-pair complex.

![Fig. 7. Determination of the mole ratio of ISO:TB via Job’s method of continuous variation](image)

**Calibration curve**

A linear calibration curve was built for the colorimetric determination of ISO in a pharmaceutical composition under optimum conditions for the formation of the ion-pair complex of ISO with TB. The resultant calibration curve excellent agreement to beer’s law on the quantification range of 1-40μg/ml with linearity of 0.9993 (Figure 8). Nevertheless, more analytical parameters are listed in (Table 2) below.

![Fig. 8. Calibration curve for the quantification of ISO](image)

**Tab. 2. Some spectral parameters related to the regression equation for ISO determination via ion-pair formation.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>λmax (nm)</td>
<td>404</td>
</tr>
<tr>
<td>color</td>
<td>Yellow</td>
</tr>
<tr>
<td>Range of linearity (µg/ml)</td>
<td>1 - 40</td>
</tr>
<tr>
<td>Molar absorptivity (L.mol.cm⁻¹)</td>
<td>1000x408.434x0.0126</td>
</tr>
<tr>
<td>Regression equation</td>
<td>Y = 0.0126x0.0059</td>
</tr>
<tr>
<td>Correlation of Linearity (R²)</td>
<td>0.9993</td>
</tr>
<tr>
<td>Calibration Sensitivity</td>
<td>0.0126</td>
</tr>
<tr>
<td>Sandells Sensitivity (µg.cm⁻²)</td>
<td>0.0793</td>
</tr>
<tr>
<td>LOD (µg/ml)</td>
<td>0.714</td>
</tr>
<tr>
<td>LOQ (µg/ml)</td>
<td>2.380</td>
</tr>
</tbody>
</table>

**Method validation**

The accurateness of the suggested method was confirmed by three replicate analyses, i.e. n=3. Three different concentrations of ISO were used (10, 20, 30 µg/ml) to calculate the method recovery and the relative error percentage, as shown in (Table 3). The results were satisfactory as indicating the high accuracy of the method. The precision was expressed as a function of the “relative standard deviation” (RSD %) which found to be within the range of (1.1938-0.5954).

**Tab. 3. Estimation of accuracy and precision of the suggested method.**

<table>
<thead>
<tr>
<th>Concentration of ISO (µg/mL)</th>
<th>Recovery percentage</th>
<th>Relative Error %</th>
<th>R.S.D. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taken</td>
<td>Found (n=3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>10.140</td>
<td>101.4</td>
<td>1.4</td>
</tr>
<tr>
<td>20</td>
<td>19.478</td>
<td>97.4</td>
<td>-2.61</td>
</tr>
<tr>
<td>30</td>
<td>29.531</td>
<td>98.4</td>
<td>-1.56</td>
</tr>
</tbody>
</table>
Interference studies

Under optimum experimental conditions, the influence of several foreign constituents which may be existing in medicinal products and may have an impact on the reaction of ISO with TB were examined. The tests indicated that common excipients like in the case of glucose, sucrose, lactose, and starch do not interfere with the colorimetric analysis of ISO even when they present in a high concentration of 1000 μg/ml (Table 4).

Tab. 4. The recovery percentage of 20μg/ml of ISO in the presence of 1000μg/ml of excipients.

<table>
<thead>
<tr>
<th>Excipient present (1000μg/ml)</th>
<th>ISO initial Conc. (20 μg/ml)</th>
<th>ISO Conc. Found (μg/ml)</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose</td>
<td>19.769</td>
<td>19.769</td>
<td>98.849</td>
</tr>
<tr>
<td>Sucrose</td>
<td>19.928</td>
<td>19.928</td>
<td>99.642</td>
</tr>
<tr>
<td>Starch</td>
<td>20.325</td>
<td>20.325</td>
<td>101.626</td>
</tr>
<tr>
<td>Glucose</td>
<td>19.531</td>
<td>19.531</td>
<td>97.658</td>
</tr>
</tbody>
</table>

Analysis of dosage forms

The good validation of the method outcomes has made the suggested method suitable for quality control analysis of ISO in commercial tablets (Table 5).

Tab. 5. Determination of Isopropamide iodide in pharmaceutical formulations (n=3)

<table>
<thead>
<tr>
<th>Pharmaceutical formulations</th>
<th>Label amount (mg)</th>
<th>Found by proposed method (mg)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablet</td>
<td>5mg/tab</td>
<td>5.00</td>
<td>100.1</td>
</tr>
</tbody>
</table>

Conclusion

A spectrometric methodology has been established and validated for the quantification of Isopropamide iodide (ISO) in pure form and as a medicine. The method has a number of privileges over other instrumental techniques of ISO analysis. It is affordable, especially when compared to chromatographic techniques, as well as rapid, and highly accurate. Moreover, common excipients did not interfere with the proposed procedure. For the prementioned, this method is highly recommended for routine analysis of ISO and may apply for other active pharmaceuticals with a similar functional group.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Chemistry Department and all experiments were carried out in accordance with approved guidelines.

References


Effects of Sulfonylureas Treatment on Apoptosis, Total Superoxide Dismutase activity and Some Biochemical Parameters in Type 2 Diabetic Patients

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Abstract

The purpose of this analysis was to evaluate the influence of anti-diabetic drugs (sulfonylureas) in type two diabetic patients and the risk of developing diabetes complications related to kidney disease (Diabetic Nephropathy) compared to healthy subjects by measuring influence of some apoptosis factors (serum Tumor necrosis factor soluble Receptor-1(TNF-R1) and TNF-related apoptosis-inducing ligand receptor-2 (TRAIL-R2)) concentrations, serum Small mothers against decapentaplegic (Smad1) as anew marker of diabetic nephropathy (DN), Superoxide dismutase concentration (SOD), Glycated hemoglobin (HbA1c%), Fasting plasma glucose (FPG), insulin concentration, insulin resistance (IR), urea and creatinine. A samples of convenience composed of 17 type 2 diabetic patients with sulfonylureas monotherapy and 17 normal subjects above the age of 40 years were recruited. Age, duration of disease and body mass index (BMI) was created. The mean levels of Smad1 and TRAIL- R2 in diabetics with sulfonylureas treatment, they were significantly higher compared to control subjects, while the means of SOD have low level in diabetic subjects, and the mean levels of TNF-R1 and IR were non-significant in diabetic with Sulfonylureas treatment compared to control subjects.

Keywords: T2DM, TNF-R1, TRAIL- R2, Smad1, SOD, Sulfonylureas.

Introduction

Diabetes mellitus (DM) was a metabolic disorder in which the body is unable to manufacture or use insulin adequately. Type 2 diabetes (T2DM) is a heterogeneous syndrome that was related to both defective secretion of insulin and insulin resistance(1). Poorly controlled levels of blood glucose usually lead to different complications in microvascular organs, such as nephropathy. (2) Diabetic nephropathy (DN) was a vascular disease risk factor and was widespread among T2DM patients (3). The request for more very reliable sensitive and specific biomarkers was needed for early expectation the progression and beginning of DN (4). Small mothers against decapentaplegic (Smad) signaling was Has been known as a main route of transforming Growth Factor-β (TGF-β) signaling in progressive fibrosis of renal (5). The Smad family of proteins was classified into three classes: Receptor-regulated Smads R-Smad (Smad1, Smad2, Smad3, Smad5, and Smad8), co-Smad (Smad4), and Inhibitor Smads I-Smad (Smad6 and Smad7).(6)

Smad1 was novel biomarkers in serum and urine for kidney injury associated with DN it can be used in the early diagnosis of DN or in the valuation of the treatment to prevent (7). Hyperglycaemia induces increase free radical formation (8). A family of antioxidant enzymes were superoxide dismutase (SOD) that control reactive oxygen species (ROS) levels by catalyzing of the superoxide radical conversion to hydrogen peroxide and oxygen molecule (9). Apoptosis was the natural mechanism of the cell for programmed cell death, it plays a vital role in both growth and homeostasis (10). Apoptosis can be induced by intrinsic pathways that are caused by cellular stress and DNA damage or by extrinsic signals that trigger death receptors on the cell surface. Such as TNF receptor 1 (TNFR-1) and ligand receptor 2 (TRAILR-2) linked to apoptosis-inducing TNF. (11)

Most patients with T2DM ultimately require pharmacologic glucose-lowering therapy, with a goal of decreasing long-term complications.(12)
Sulfonylureas have an excellent blood glucose reducing effect; sulfonylureas was an effective drug for diabetes treatment and hyperglycemia (13). The current study was conducted to investigate the effect of Sulfonylureas as a monotherapy in diabetic patients on the release of soluble death receptor-activated apoptosis (TNFR/R1 and TRAIL/R2), study the effect of Sulfonylureas drugs on activity of Smad1 in serum as a novel biomarker for early detection and evaluation of diabetic nephropathy severity in patients with T2DM (for the first time measured in blood serum) and Study the impact of Sulfonylureas on stress from oxidation by determination the activity of superoxide dismutase (SOD). Thus to explore the effect of this treatment on the risk of developing diabetes complications related to kidney disease.

Materials and method

At the National Diabetes Centre / AL -Mustansiriya University thirty four (34) of T2DM patients and healthy subjects were chosen. The patients samples were divided to two groups. Group1 consists of 17 Sulfonylureas therapy taking patients and the group 2 was consists of 17 apparently healthy individual (control group ). Patients with other diseases, smoking patients and pregnant female patients were excluded from the medical assessment study. Patients that take other than metformin drugs or metformin plus insulin had also been excluded. he blood sample was split into two aliquots; the first aliquot blood was delivered to a tube comprising Ethylene Diamine Tetraactic Acid (EDTA), this blood was gently mixed and then used to measure HbA1c, while the second aliquot was delivered to the gel plain tubes to extract serum. Control and patients subjects were subjected to determination of the tests: serum insulin hormone was measured by ELISA using kit (Sandwich) supplied by Cobas (ROCHE), the Homeostasis Model Assessment (HOMA) assessed insulin resistance (IR), SOD was determined in serum by (riboflavin/NBT method), TNF/R1 was measured using ELISA kit (Sandwich) supplied by Cusabio (USA), serum TRAIL/R2 was measured by using Sandwich ELISA kit provided by Cusabio (USA), serum Smad1 was measured by using ELISA kit (Sandwich) supplied by Mybiosource (CANADA), HbA1C % was measured using the Integrated Sciences (Australia) A1C test system, fasting plasma glucose concentration, serum urea and serum creatinine were estimated by colorimetric method by using a kit supplied by Bio Systems (SPAIN).

Statistical Analysis

Statistics was performed with T-Independent test for difference between two groups, the result were expressed as mean ± SD, different range test at p ≤ 0.05 which was agreed as significant, where as highly significant when p ≤ 0.001, and the range test at p ≥ 0.05 was agreed as non-significant.

Results & Discussion

Demographic data for (17) patients with T2DM and (17) healthy subjects, the result in Table (1) showed that the Mean± SD value for study parameters, and Compare between different groups in current study.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameter</th>
<th>G1=17 sample</th>
<th>G1=17 sample</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diabetic with Sulfonylureas therapy</td>
<td>Healthy control group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>58.52±7.55</td>
<td>51.88±8.73</td>
<td>*0.032</td>
<td></td>
</tr>
<tr>
<td>Duration (years)</td>
<td>7.82±4.77</td>
<td>0.000</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>BMI (Kg/m2)</td>
<td>28.51±2.84</td>
<td>30.19±2.23</td>
<td>0.203</td>
<td></td>
</tr>
<tr>
<td>F.S.G.(mg/dl)</td>
<td>200.58±53.81</td>
<td>92.23±11.33</td>
<td>*0.000</td>
<td></td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>8.89±2.12</td>
<td>4.69±0.61</td>
<td>*0.000</td>
<td></td>
</tr>
</tbody>
</table>
Table (1): Distribution of mean± SD for all variables and parameters in all Studied Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients Group Mean± SD</th>
<th>Control Group Mean± SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (µU/mL)</td>
<td>12.49 ± 6.25</td>
<td>11.73 ± 4.27</td>
<td>0.240</td>
</tr>
<tr>
<td>IR</td>
<td>2.07 ±1.03</td>
<td>1.327±0.188</td>
<td>0.310</td>
</tr>
<tr>
<td>S. Urea (mg/dL)</td>
<td>29.54±6.84</td>
<td>23.7824±3.44</td>
<td>*0.045</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.60±0.16</td>
<td>0.58±0.10</td>
<td>0.432</td>
</tr>
<tr>
<td>Smad 1 ( ng/mL)</td>
<td>1.244±0.315</td>
<td>0.144±0.026</td>
<td>*0.000</td>
</tr>
<tr>
<td>SOD (U/ mL )</td>
<td>109.68±13.65</td>
<td>151.91±11.64</td>
<td>*0.000</td>
</tr>
<tr>
<td>TNF-R1 (pg/mL)</td>
<td>0.563±0.205</td>
<td>0.555±0.220</td>
<td>0.392</td>
</tr>
<tr>
<td>TRAIL-R2 (pg/mL)</td>
<td>0.48±0.12</td>
<td>0.28±0.07</td>
<td>*0.00</td>
</tr>
</tbody>
</table>

* Significant using ONEWAY-ANOVA at 0.05 level

The results of age have shown that there were highly significant difference among the study groups. These results were due to the duration of the disease for the patient group. Note that all samples and healthy control were chosen above the age of 40 years old. Body mass index BMI showed no significant differences (P=0.203) between patients group, when they were compared to control group.

The statistical distribution of mean for glycaemic state (FPS, HbA1c, Insulin Level, and HOMA IR) were performed on all studied groups generally as shown in Table (1). Results of fasting glucose in plasma (FPG) expressed in mg/dl revealed that there were high significant variations between patients group when compared to healthy control group (P<0.0001). The results of glycated hemoglobin (HbA1c %) levels showed higher significant increasing in patients group (P<0.0001), when compared to the control healthy subjects. In current study, a high level of HbA1c and FPG were found in patients group, which identifies to poorly controlled glycemia in these subjects. These results because of treating with Sulfonylureas he initial excellent response resulted in a loss of active antidiabetic response and induce βcell failure (secondary failure) (14). The results of serum insulin levels (µU/mL) for patient groups showed that there were no significant differences between patients group when compared with control group. The same results have been observed in the insulin resistance levels in studied groups which indicated that there was no significant differences between patients group compared with control healthy subjects. The results of insulin and insulin resistance in our study were connected and intertwined. The results of current study were agreed with previous studies which explained that sulfonylurea drugs, showed some evidence to reduce insulin resistance in addition to its insulin secretory action. (15) Mean± SD for renal function tests (Urea, and Creatinine) were performed on all studied groups as shown in Table (1). The result of urea concentration (mg/dl) significant differences are observed in it is level of patient group compared to control healthy group (P=0.045). All results are within normal values, Previous studies were showed that there was statistically significantly elevated levels of urea with increased levels of blood sugar and a strong connection between blood urea and blood sugar. (16)

Serum creatinine is a more accurate evaluation of renal function than urea, but urea has been increased in renal disease earlier. (17) The results of creatinine concentration (mg/dl) showed no significant differences are found in it is level of patients group compared to control healthy group. Serum urea and creatinine measures have been readily available tests that assist in
early diagnosis and avoidance of diabetic kidney disease and can restrict development to end-stage renal disease. (18) Smad1 were novel reliable biomarkers for each of the classical pathological finding in the beginning periods of DN and were indicator of progressive glomulare filtration rate decline (19). The results of the Serum Smad1 concentration (ng/mL) which was measured for the first time in the blood serum, showed that there were highly significant differences between patients groups and healthy control subjects (P<0.0001). The mean± sd of Smad1 concentration in diabetic patients with sulfonylureas treatment was found to be significantly elevated compared with healthy subjects group, this results revealed that using of sulfonylureas as monotherapy in diabetic patients does not reduce the risk of high Smad1 concentration. The effect of sulfonylureas treatment was not recorded in previous studies, recent studies found The Smad1 is the primary signaling molecule directly concerned in DN and other kidney disease induction and development of glomerulosclerosis (20). The results of the serum total SOD levels (U/mL) in T2DM patients and control groups showed that there were highly significant differences between studied groups (P<0.0001). The mean± sd of SOD concentration in control group (151.91±11.64) was significantly increased compared with patients group (0.844 ± 0.285). This finding was generally consistent with literature data and was a clear indicator of the presence of oxidative stress in people with diabetic. Pervious study showed that hyperglycaemia in diabetes stimulates different biochemical processes leading to an increase superoxide and hydroxyl radical formation, which may lead to reduced n SOD enzyme activity. (21), While some have not documented any changes(22) And in diabetic patients, few researchers found elevated serum SOD enzyme activity(23). Recently it was shown that death receptors can be established in a soluble form in the bloodstream (24). Levels of serum (TNF-R1) concentration (pg/mL) for studied groups showed that there were not significant differences between patients group when compared with control group, so the result of the current study showed that using of Sulfonylureas therapy may reduce TNF-R1 concentration. This finding was consistent with literature data which know that the levels of serum TNF and its receptors were significantly decreased in diabetic patients with Sulfonylureas treatment (25). The results of TRAIL-R2 concentration (pg/mL) showed elevated significant difference between patients groups (P<0.0001), when compared to control groups. This result correspond to study which showed that a high glucose level, characteristic of diabetes was increased serum TRAIL binding to TRAIL-R1 and TRAIL-R2 levels(26). Results of apoptosis factor (TRAIL-R2), and SOD were generally consistent with literature data which showed that sulfonylureas treatment have been reported to accelerate cells apoptosis and stimulated production of reactive oxygen species (ROS) which could result in reduced activity of SOD enzyme. (27) These results may explain the high concentration of serum Smad1 in diabetic patients which indicates that patients with sulfonylureas treatment may exposure risk of developing diabetes complications related to kidney disease (Diabetic Nephropathy).

**Conclusion**

Using of Sulfonylureas as a monotherapy in T2DM patients did not reduce the risk of developing diabetes complications related to kidney disease (Diabetic nephropathy).

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Department of Chemistry, College of Sciences for Women, University of Baghdad, Baghdad-Iraq and all experiments were carried out in accordance with approved guidelines.

**References**


An analytical Study of the Maximum Force Variable on the walkway of the (100, 1500 and 5000 m Sprinters For Applicants)

Assist. Prof. Dr. Haider Fayyadh Alamiri
University of Kufa, Physical Education of Sport And Science

Abstract

The Motion analysis is used to solve problems which related to the learning and training. In addition, It diagnoses movements, compares their parts, times and strengths, and compares between good and bad movement, as well as it helps to develop the movement and its performance. There are few studies in of motion analytical for study. The researcher design this study in a scientific procedure by using the scanner device (Gait Analysis) of the German company (Zebris) to read the data in a standardized manner during the test, the researcher selected the sample of the Iraqi champions in the competition of (100 m and 1500 m and 5000 m) Two for each contest in a deliberate way for the heroes of Iraq and the number of them six, two for each contest, for the Time period from 11/9/2018 to 1/12/2018, in the laboratory of physical mechanics in the Faculty of Physical Education and Sports Sciences / University of Kufa. The search variable was chosen maximum force because it has a large role in the movement and transfer of athletic during the race, Concluded researcher to The maximum amount of force distributed on the footprint (front, center and heel) and according to the requirements of the race, and The higher the speed and force.

Keywords: Biomechanical indicators, print foot, analysis

Introduction

Whether using theoretical or applied mathematical sciences or scientific methods and modern techniques, has developed the analysis of the movement of a large extent recently for several reasons, Modern techniques, which have helped to determine the variables of kinetics of athletes accurately, is a dynamic analysis of the important science, which relies primarily on the use of laws and foundations used in the science of biomechanics for the study of free. This development was positively reflected in the level of determining the critical points in performance and in the various sporting events, especially those running short, medium and long distances, which are the activities of athletics, which rely heavily on the level of mechanical performance. Jogging and physical abilities such as strength, speed, lengthening, endurance, explosive force. The natural movements of the person and the athletics competitions is a “set of individual races that include at the same time racing relay. The performance of the members of the team and this is a combination of individual and collective so as to give confidence to the contestant and the viewer alike. The Muscle strength is one of the necessary physical attributes of the various games and motor skills on which the achievement and its requirements depend. It means the ability or tension that the muscle group can produce against resistance at the maximum voluntary contraction. Motion analysis is used to solve problems which related to the learning and training. In addition, It diagnoses movements, compares their parts, times and strengths, and compares between good and bad movement, as well as it helps to develop the movement and its performance. There are few studies in of motion analytical for study. The variable of maximum force between the runners of the races (100 m, 1500 m and 5000 m) in Iraq, for that The researcher design this study in a scientific procedure by using the scanner device (Gait Analysis) of the German company (Zebris) to read the data in a standardized manner during the test. The present study aimed to identifying the variable values of the race (100 m, 1500 m and 5000 m) for the Iraqi champions at a speed of 10 km, 16 km and 22 km and Comparison of these variable values in each competitions for the heroes Iraqi at the speed of (10 km, 16 km and 22 km).
Material and Method

The researcher used descriptive analytical method to process data and information related to the nature of the problem. The method research causality is the research in which the researcher tries to determine the cause of differences in the behavior or condition of a group of individuals, which means that the observed groups vary among themselves in the attribution of some variables\textsuperscript{12, 13}. And the knowledge of similarities and differences requires access to information related to this phenomenon “The purpose of organizing this information and classification is to help the researcher to reach conclusions and generalizations that help us to develop the reality we are studying, the descriptive method which aims to reach conclusions that contribute to understanding this reality and development\textsuperscript{14, 15}. The researcher selected the sample of the Iraqi champions in the competition of (100 m and 1500 m and 5000 m) Two for each contest in a deliberate way. for the heroes of Iraq and the number of them six, two for each contest. for the Time period from 11/9/2018 to 1/12/1018. in the laboratory of physical mechanics in the Faculty of Physical Education and Sports Sciences / University of Kufa. The search variable was chosen maximum force because it has a large role in the movement and transfer of athletic during the race, The test was held at the Bio Mechanical Laboratory on 11/9/2018 at 4:00 pm in the presence of the auxiliary team and the contestants 100m, 1500m and 5000m. The speed at which the test was conducted was determined to the maximum force the runner could play during running. the selected was a speed of 10 km / h on the device for race (5000 m) and the speed of 16 km / h for the race (1500) and the speed of 22 km / h for the distance of race (100 m). The choice of these speeds was in accordance with the times achieved by the contestants in the official competitions, By dividing the total distance on the time to extract the rate of speed (meters / second) and then be multiplied by (3.6) to convert to kilometers, because the speed on the device walking platform dealing with the kilometer, the device of gait analysis has a different speed starting from ( 1 km to 22 km) per hour, as well as that the device is able to extract more than fifty variables for both feet (left And right) so which the use of these variables in the identification of errors and the development of appropriate exercises to them, then the rise of contestants on the gait analysis device,( the platform walking and running) The device doing when the runner up to the speed required for each runner\textsuperscript{16}, then The data is recorded by the cable contact with the platform to the calculator that includes the application of the platform (zebris FDM).Then the recording on device takes 10 seconds to obtain the data during the test.

Results

After extracting the data obtained by the researcher from the test in relation to the variable the maximum force of the contenders for distance (100 m, 1500 m and 5000 m) which related to the research and processing statistically by extracting the mean and standard deviation, then the researcher presented the results reached and Analyze and discuss them and then interpret indicators of achieve

<table>
<thead>
<tr>
<th>Variable name</th>
<th>right</th>
<th>The speed 10 km/h</th>
<th>The speed 16 km/h</th>
<th>The speed 22 km/h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mean SD</td>
<td>mean SD</td>
<td>mean SD</td>
</tr>
<tr>
<td>left</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max force, N</td>
<td>L</td>
<td>979.4</td>
<td>42.28</td>
<td>1104.8</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>993.1</td>
<td>67.88</td>
<td>1109.4</td>
</tr>
<tr>
<td>Midfoot</td>
<td>L</td>
<td>360</td>
<td>2.89</td>
<td>417.0</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>343.</td>
<td>47.87</td>
<td>388.6</td>
</tr>
<tr>
<td>Heel</td>
<td>L</td>
<td>303.7</td>
<td>43.1</td>
<td>462.4</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>362.2</td>
<td>2.54</td>
<td>400.8</td>
</tr>
</tbody>
</table>
Table 1 shows the arithmetic mean and the standard deviation of the biomechanical variable. The maximum force of the feet (right-left) of the competitors (100 m, 1500 m and 5000 m) of the six runners for each contest two and used three speeds on the gait analysis 10 km for the contestants long competitions and the speed of 16 km for the competitions medium and the speed of 22 km for fast competitions, as the footprint of the contestants are measured for three areas of the foot (forefoot - the midfoot - the heel foot) and the left and right.

The researcher found that attributed the reason for this difference to the nature of the competitions in terms of distance and the mechanical of jogging for the runners on the feet, additionally the nature of the competition imposing the runner to put the foot on the ground, as well as the amount of payment made by the runners so that the impulse force to the runners varies depending on the nature of the competition, as the amount of impulse depends mainly on the maximum force and contact time, the greater the amount of force (impulse force = force + time), and therefore the runner spend force during it exerting and according to the nature of the distance. The difference in the strength of the runners is observed through the analysis of the right and left sides of the footprint and the Values of the force variable as well as forms of force curve schemas in each part of a second, as shown in Figure 1.

![Figure 1: force curves](image)

Table 1 shows that there is a difference between the maximum strength of the front foot and the left and right sides at the same speed for the contestants, whether run (10 km), (16 km) or (22 km). The researcher attributes the difference between the right and the foot left to the same rider is for the presence of a favorite man to the contestant or that the contestant uses a leg more than the other, during the jump or take off, which makes that leg has a greater force than the other it and shows that when running on the gait analysis device so that read the data for the footprint left or right where the difference them it, as shown in Figure 2.
Looking back at Table 1 to analyze the values of the maximum force of the heel of the foot between the speeds of the contestants, as shown in Figure 3.

**Discussion**

The spindle of the foot is the foot joint and therefore the farther of the point of impact of the force from the axis of rotation will increase the torque of the force 17, which explains that the sprinters short distance running dependent on the front of the foot any comb and thus will be the point of impact of the strength of the short distance runner at the farthest point of the front of the foot18, 19, which helps to obtain the torque of a large force as well as generating large torque during the course of pushing the foot from the ground and get a likely background large resulting from the rotational momentum when the center of gravity of the body in front of the so-called focal of back 20, 21, and this weighted will help the work weighted Front with Good standing when the center of gravity of the body behind the focal point 22, 23, which is called the front of built upon.

As for the mid-distance sprinters, they will be after the point of influence of the force on the axis of rotation less than the fast-distance runners because they do not need a great torque to generate to swing the leg faster 24, 25, because for the length of the race distance also will lead to the fatigue of the contestant quickly26.

As well as for the long-distance runners will be after the point of the impact of power on the axis of rotation less than the runners of high-speed and medium because they do not need a great torque to generate a torque to swing the leg faster and for the length of the race distance.
also will lead to the fatigue of the racer quickly 27, 28, Almost runners a full footprint puts on the ground is required for the mechanical performance of jogging long distances.

**Conclusion**

The maximum amount of force exerted by short, medium and long distance runners varies because of the different race distance, and the maximum amount of force distributed on the footprint (front, center and heel) and according to the requirements of the race, and the higher the speed and force, the greater the pressure and the it will less space for the base of the footprint, and the maximum amount of force plays a big role in achieving the achievement for the short, medium and long distance runners and all according to its purpose.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the University of kufa, Iraq and all experiments were carried out in accordance with approved guidelines.

**References**


An optimized Retina Recognition Technique Based on PSO Inspired Algorithm

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Abstract

Retina Recognition techniques capture and analyze the vascular patterns on the thin nerve on the backs of an eyeball which process light getting in the pupil. Retinal patterns are extremely distinguishing traits; it cannot be gotten or stolen since there are no two persons have similar patterns of retinal, as well as the features extracted from the retinal image are the most stable and reliable than other biometric features. Therefore, the retina is selected as a robust source in the proposed recognition techniques. In this paper, an optimized technique of retina recognition based on an inspired algorithm is proposed for the authentication purpose in which particle swarm optimization (PSO) inspired algorithm is utilized for extracting features from retinal images. The obtained results from this work declared that the PSO provides an optimized performance with 100% accuracy and 3.5 seconds time-consuming.

Keywords: An Optimized Recognition Technique, Retina Patterns, PSO Inspired Algorithm.

Introduction

The biometric recognition techniques for the purpose of authentication like face detection, retina, iris, and fingerprint scanning are more securely than other traditional authentication techniques like password, PIN, and etc. In these traditional authentication techniques, the users should remember the password unique combination. Furthermore, the password should be confidential since this is the main objective of the system to be secure. Additionally, the password and PIN-based techniques can be attacked by brute force dictionary or by guessing 

Unlike traditional techniques, Biometric authentication techniques give better usability and reliability since it need not be remembered. Generally, there are two types of biometric recognition techniques; physiological like retina scanning, and behavioral like voice, and Handwritten Signature.

Retina authentication is the most promising biometric technique comparing to other biometric techniques. The retina remains unchangeable during the lifespan of the person. Furthermore, retina exists inside the eye that makes it cannot be forged owing to its internal protective position which prevents it from deformation. The blood vessels patterns in the retina have given a high level of security and uniqueness (even in identical twins) which is the foundation of retina recognition. In addition to the blood vessels, the regions of the fovea and optical disk are two essential properties in the retina, figure 1 illustrates the retina regions.

Figure 1: The image of retina regions.

The inspired algorithms have become an essential research field owing to intent to simulate nature for solving the complex real-life issues, especially in optimization. Particle swarm optimization (PSO) inspired algorithm has been utilized in this work for extracting optimal features from human retina image.

This paper is structured as follows; the related works are presented in the next section; Third section explains...
the PSO inspired algorithm; Fourth Section presents the proposed optimized retina recognition technique; Fifth section explains the experimental results; The last section, presents the conclusions of this work, furthermore, it provides suggestions for future work. This technique provides an average identification rate of 92.50. Mahrokh Khakzar and Hossein Pourghassem 4 , presented a retina authentication technique based on extracting the features from the spatial graph of the vessel pattern. The retina images were enhanced utilizing the preprocessing phase, and the retina vessel skeleton of this enhanced image was extracted utilizing the Isotropic undecimated wavelet transform algorithm. For generating a thin vessel and obtaining the bifurcations and vessel ends, the morphological operator has been utilized. The spatial graph adjacency matrix combining with the vessel patterns were generated automatically utilizing the relational mathematical graph (RMG) algorithm. After that, considerable features depending on the theory of spectral graph were used for developing the matching process. The obtained results showed that the proposed technique achieves a 97.14% accuracy rate. A. Saha et al. 9 , presented a multi-modal individual authentication technique based on iris and retina recognition for providing a high degree of distinction, universality, and circumvention. To minimize the main issue related to feature level fusion, the Principal Component Analysis was used. The rate of recognition for this technique was 96.74% and 94.56% for iris and retina recognition respectively.

1. **PSO Inspired Algorithm**

PSO is presented by Kennedy and Elberhart 10, inspired by social behavior of bird flocking or fish schooling. The PSO algorithm searches the space of a fitness function by adjusting the individual agent’s trajectories, named particles, as the piecewise paths created via positional vectors in a quasi-stochastic way. The swarming particle movement includes two main components: stochastic and deterministic components. Every particle is attracted toward the location of the current global best and its own best location in history, but, at the same time it has a tendency for moving randomly. If a particle gets a position which is best than any formerly found positions, updates that position as the new current best for particle 11.

In every iteration, a loop in the program is determined to every agent that another agent was its nearest neighbor, then assigned that agent’s X and Y velocities to the agent in focus. As it is predictable, it has been viewed that solely utilize such a strategy will quickly settle down the swarm on a unanimous, unchanging direction. For avoiding this, a stochastic variable named craziness was presented. In every iteration, several changes were added to randomly chosen X and Y velocities. This presented enough variation into the system to give the simulation a “life-like” appearance. The previous explanation illustrates the most needed features of PSO that refers to its seemingly unalterable nondeterministic nature: incorporation of randomness.

The conception was to allow the birds (agents) to get an unknown favorable location in the search space (food source) via capitalizing on one another’s knowledge. Every agent could remember its best position and knowing the best position of the whole swarm. The extreme of the mathematical function to be optimized can be thought of as the food source. After a sequence of minor alterations and elimination of the ancillary variables, the rules of updating to calculate the next location of a particle were presented as Equation 1 and Equation 2.

\[
\begin{align*}
\mathbf{v}_i &= w \mathbf{v}_i + c_1 \mathbf{r}_1 \cdot (\mathbf{p}_i - \mathbf{x}_i) + c_2 \mathbf{r}_2 \cdot (\mathbf{p}_g - \mathbf{x}_i) \quad \text{(1)} \\
\mathbf{x}_i &= \mathbf{x}_i + \mathbf{v}_i \quad \text{(2)}
\end{align*}
\]

Where \( \mathbf{x}_i \) and \( \mathbf{v}_i \) are the \( i^{\text{th}} \) particle’s position and velocity vector, respectively; \( \mathbf{r}_1 \) and \( \mathbf{r}_2 \) are two random numbers; \( \mathbf{p}_i \) and \( \mathbf{p}_g \) represent the best positions experienced so far by the \( i^{\text{th}} \) particle and the whole swarm, respectively; and \( c_1 \) and \( c_2 \) are two parameters indicating the particle’s confidence in itself (cognition) and in the swarm (social behavior), respectively. These two parameters are among the most important parameters of the algorithm in that they control the balance between exploration and exploration tendencies. A relatively high value of \( c_1 \) will encourage the particles to move toward their local best experiences, while higher values of \( c_2 \) will result in faster convergence to the global best position 12 . PSO inspired algorithm is shown in Algorithm 1.
Algorithm 1: PSO Inspired Algorithm

for p=1 to numbers of birds do
if M(ki) > M(si) then /* M ( ) evaluate strong*/
for f=1 to number x do
sid = Qid /*Qid is the strong find so far*/
ends
m=p /*arbitrary*/
for p=index neighborhood do
if M(pj) > M(pg) then
e=p
ends
ends
for p= 1 to num of dim do
refresh depend to Eq. (1)
refresh place depends to Eq. (2)
ends
ends
when stop
ends

2. The Proposed Retina Recognition Technique

This section describes the design and implementation of the proposed optimized retina authentication technique using PSO inspired algorithm. This proposed technique includes training and testing phases, every phase includes three processes; Firstly, the preprocessing; Secondly, the feature extraction; And thirdly, the matching. These phases include the same preprocessing and feature extraction processes but differ in the matching process. The general block diagram of the proposed technique for the training and testing sections are shown in figure 2 and figure 3.

Figure 2: Block diagram of the proposed technique training section.

2.1 Preprocessing

This process includes several steps as follows;

Step1: Apply the contrast image enhancement process to the input retina image (as in Algorithm 2) for enhancing the retina image and obtaining additional specifics to the vessel edges. This step improves the objects perceptibility via enhancing the brightness difference between the object and their background.

Algorithm 2: Contrast Image Enhancement Algorithm

Input: Mean_Img //Image after local mean compensation process
Mask_Img///Mask image, H, W /// The image Height & Width, respectively
Output: Con_Img ///Resulting image [0,1]

Figure 3: Block diagram of the proposed technique testing section.
Step1: Calculate mean and standard deviation values (STD)
Set Mean ← 0: STD ← 0: N ← 0 // Initial values
If Mask_i Then Set Mean ← Mean + Mean_Img(x,y)
Set N ← N+1
End If
End For
Set Mean ← Mean/N
If Mask_1 Then
Set STD ← STD+Power((Mean_Img(x,y)- Mean),2)
End For
Set STD ← Sqrt((1/ N) *STD)

Step2: Calculate minimum (Min) and maximum values (Max)
Set alpha1←1.1 ///Predefined value used for computing minimum value
Set alpha2← 0.3 ///Predefined value used for computing maximum value
Set Min ← Mean - (alpha1×STD)
If Min<0 Then Set Min ← 0
Set Max ← Mean +(alpha2×STD)
If Max>255 Then
Set Max ← 255
Set Gam ← 6.5 ///Predefined value
Step3: Calculate new values from old values
For all y,x Do
If Mean_Img(x,y) ← Min Then Set Con
Else If n← Max Then Set
Else Set × ((Mean_Img(x,y) - Min)/(Max - Min))
End If
Else Set Con_Img(x,y) ← 0
End For
For all y,x Do {where m} ///Normalization values
If Con >0 Then Set Con_Img(x,y) ←1
End For
Step4: End

Step2: Apply the canny filter for detecting and recognizing the vessel edges.

Step3: Transform the obtained image from the canny filter using the discrete wavelet transform to get four sub-images (LL, HL, LH, HH). LL is selected to be used in the next process.

2.2 Feature Extraction Process

In this process, a set of distinguished information is extracted from the resulted preprocessed image. This information refers to the required features vector for distinguishing among persons. In the proposed recognition technique, the PSO inspired algorithm is utilized for extracting the features from the retina image.

Feature in the PSO inspired algorithm was extracted according to the movement of bird’s direction. The implemented PSO algorithm for features extraction is explained in the following steps:

Step1: Choose the number of iterations.

Step2: Choose the number of particles.

Step3: Detect the center of the retina to detect the distance of the particle from the center.

Step4: Release the particle to the retina image to find the best position.

Step5: Find the best position by using guide particle.

Step6: Make the guide is the best particle.

Step7: Repeat the process with another iteration.

Step8: Collect all the features with (position (i,j), and the distance of the features from the center of the retina.

Step9: Save the features vector in the PSO database.

Note that for each iteration in the PSO algorithm, there is a new best position will be found by the particle, so, the guide particle will modify itself depending on the new best position. Then the feature will be saved in the PSO database.

2.3 Matching Process

The matching is an essential process for any biometric recognition technique which is utilized for finding the similarity degree between two patterns of the retina, where the distance between the features vector of the template stored in the Database and the tested image is computed. The measure utilized in this proposed technique is the Sum of Squared Difference (SSD).

3. The Experimental Results and Tests

The proposed retina recognition technique was implemented using VB.NET programming language. For testing the performance of the optimized technique, the Digital Retinal Images for Optic Nerve Segmentation Database (DIRONS-DB) has been utilized. This dataset includes 110 images, 100 for training and 10 for testing, each image of size 565×584 of (24 bit/pixel). Figure 4
shows some of the retina image samples belong to the used dataset.

![Figure 4: Some samples of retina images from DIRONS-DB.](image)

The preprocessing gives a certain result that deal with enhancement of the retina image to find the best clear resolution of the blood vessels and optic disc that prepare for feature extraction. In order to detect the blood vessels with high clarity, the contrast filtering must be done. Table 1 shows the effect of contrast filter on retina images.

**Table 1: The effect of Alpha Contrast on some retina images.**

<table>
<thead>
<tr>
<th>Image</th>
<th>α</th>
<th>Contrast Filter</th>
<th>Histogram</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
<td><img src="image" alt="Contrast Filter" /></td>
<td><img src="image" alt="Histogram" /></td>
</tr>
<tr>
<td>image_015</td>
<td>0.5</td>
<td><img src="image" alt="Contrast Filter" /></td>
<td><img src="image" alt="Histogram" /></td>
</tr>
<tr>
<td>image_008</td>
<td>0.5</td>
<td><img src="image" alt="Contrast Filter" /></td>
<td><img src="image" alt="Histogram" /></td>
</tr>
<tr>
<td>image_001</td>
<td>0.5</td>
<td><img src="image" alt="Contrast Filter" /></td>
<td><img src="image" alt="Histogram" /></td>
</tr>
<tr>
<td>image_027</td>
<td>0.5</td>
<td><img src="image" alt="Contrast Filter" /></td>
<td><img src="image" alt="Histogram" /></td>
</tr>
<tr>
<td>image_021</td>
<td>0.5</td>
<td><img src="image" alt="Contrast Filter" /></td>
<td><img src="image" alt="Histogram" /></td>
</tr>
<tr>
<td>image_019</td>
<td>0.5</td>
<td><img src="image" alt="Contrast Filter" /></td>
<td><img src="image" alt="Histogram" /></td>
</tr>
<tr>
<td>image_100</td>
<td>0.5</td>
<td><img src="image" alt="Contrast Filter" /></td>
<td><img src="image" alt="Histogram" /></td>
</tr>
<tr>
<td>image_079</td>
<td>0.5</td>
<td><img src="image" alt="Contrast Filter" /></td>
<td><img src="image" alt="Histogram" /></td>
</tr>
<tr>
<td>image_044</td>
<td>0.5</td>
<td><img src="image" alt="Contrast Filter" /></td>
<td><img src="image" alt="Histogram" /></td>
</tr>
</tbody>
</table>

The parameter of the main contrast is (α Alpha contrast), therefore, table 1 shows the obtained results at different values of (α Alpha contrast parameter) with contrast image and histogram image for each value. The best contrast is attained when choosing the parameter (α= 0.5).
The parameters of canny filter process ($\alpha$, kernel, high threshold, and low threshold). These parameters have been preprocessed on the retina image in order to find a clear Gaussian image. Table 2 shows the obtained results at different values of ($\alpha$, kernel, high threshold, and low threshold). Table 3 shows the effect of parameters ($\alpha$, kernel, high threshold, and low threshold) on the contrasted image by using histogram for Gaussian image.

**Table 2: The Effect of Parameters ($\alpha$, kernel, high threshold, and low threshold) on the Contrast Image to find Gaussian image.**

<table>
<thead>
<tr>
<th>No.</th>
<th>Alpha</th>
<th>K</th>
<th>h</th>
<th>L</th>
<th>Image1</th>
<th>Image2</th>
<th>Image3</th>
<th>Image4</th>
<th>Image5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>3</td>
<td>9</td>
<td>4</td>
<td>Gaussian</td>
<td>Gaussian</td>
<td>Gaussian</td>
<td>Gaussian</td>
<td>Gaussian</td>
</tr>
<tr>
<td>2</td>
<td>0.8</td>
<td>3</td>
<td>10</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>5</td>
<td>12</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 3: The Effect of Parameters ($\alpha$, kernel, high threshold, and low threshold) by using Histogram for Gaussian.**

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameter</th>
<th>Image sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sigma</td>
<td></td>
</tr>
<tr>
<td></td>
<td>k</td>
<td>h</td>
</tr>
<tr>
<td></td>
<td>Imag1</td>
<td>Imag2</td>
</tr>
<tr>
<td>1</td>
<td>0.5</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>0.8</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>5</td>
</tr>
</tbody>
</table>

The extracted features of the PSO inspired algorithm are shown in table 4, as red dots. However, table 4 shows the results of the histogram for the extracted features depend on the best parameter in the technique (contrast $\alpha=0.5$, $H=9$, $L=4$, canny $\alpha=0.5$).
Table 4: Results of Features Extraction for PSO Inspired Algorithms, and the Histogram.

<table>
<thead>
<tr>
<th>Gaussian Image</th>
<th>Wavelet Transform</th>
<th>PSO</th>
<th>Histogram</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Gaussian Image" /></td>
<td><img src="image2.png" alt="Wavelet Transform" /></td>
<td><img src="image3.png" alt="PSO" /></td>
<td><img src="image4.png" alt="Histogram" /></td>
</tr>
</tbody>
</table>

The vector set of features that extracted from the retina image will have 100 iterations so that all sections of wavelet possess (LL, LH, HL, and HH) will extract 100 feature with its distance from the center as shown in table 5.

Table 5: The features vector of PSO algorithm.
The extracted features for PSO inspired algorithm are translated into two values: mean, and standard deviation (STD). Then, these two values are being to deal with instead of a large number of features, as shown in the table 6. Table 7 shows the time consuming in different No. iteration for the PSO algorithm.
Table 6: Mean and STD values for the extracted features to three test images.

<table>
<thead>
<tr>
<th>No.</th>
<th>Mean</th>
<th>STD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30.2509387042607</td>
<td>19.9936404193108</td>
</tr>
<tr>
<td>2</td>
<td>31.2063811829574</td>
<td>18.23200661934</td>
</tr>
<tr>
<td>3</td>
<td>33.8934603909775</td>
<td>7.9964399484224</td>
</tr>
</tbody>
</table>

Table 7: Time consume in different No. iteration for the PSO algorithm.

<table>
<thead>
<tr>
<th>No. iteration</th>
<th>Time min</th>
<th>Time max</th>
<th>Time mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>00:00:2.4</td>
<td>00:00:4.6</td>
<td>00:00:3.5</td>
</tr>
<tr>
<td>200</td>
<td>00:00:4.6</td>
<td>00:00:6.6</td>
<td>00:00:5.5</td>
</tr>
<tr>
<td>300</td>
<td>00:00:7.4</td>
<td>00:00:9.4</td>
<td>00:00:8.4</td>
</tr>
</tbody>
</table>

For the purpose of evaluating the performance of the optimized authentication technique, the verification accuracy was analyzed using the false rejection rate (FRR) and false acceptance rate (FAR). Table 8 shows the FAR and FRR and Accuracy for the PSO algorithm depending on the parameter $\alpha$, so, the best sigma is (0.5).

Table 8: FAR, FRR and the accuracy versus different threshold values.

<table>
<thead>
<tr>
<th>Parameter $\alpha$</th>
<th>FAR</th>
<th>FRR</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.033</td>
<td>0.0</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>0.067</td>
<td>0.053</td>
<td>99.94</td>
</tr>
<tr>
<td>1.5</td>
<td>0.080</td>
<td>0.067</td>
<td>99.93</td>
</tr>
<tr>
<td>2</td>
<td>0.083</td>
<td>0.093</td>
<td>99.91</td>
</tr>
</tbody>
</table>

Conclusion

Among different existing biometric techniques, retina recognition is a very secure and stable since the vascular patterns are unique and it is hard to modify or copying it. In this paper, an effective optimized technique is proposed for individual recognition depending on retinal blood vessels. The tests results conducted on the Drions dataset indicated that the performance of the proposed technique is very high with 100% recognition rate.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Ababil high school-Al-resaffa education and all experiments were carried out in accordance with approved guidelines.

References


Role of TNF alpha and IL-6 in Inflammatory Process after Tooth Extract in Children Under 12 Years

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Abstract

A cross-sectional study was carried out in Kirkuk city from November 2018 to March 2019. The number of children after tooth extraction under study were 55 children ages were between 5-12 years old. These patients admitted to private clinics of dentists. The control group who were matched to the children with tooth extraction studied, included 35 healthy children. Three ml of blood was collected by vein puncture using Vacutainer tubes from each patient enrolled in this study for determination the level of IL-6 and TNF-α by using ELISA technique. The study showed that the highest mean level of TNF-α was found in children after tooth extraction comparing with healthy control group (36.8±13.4 v.s. 13.5 ±2.9 pg/ml) (P: ≤0.05). The highest mean level of IL-6 was found in children after tooth extraction comparing with control (88.1±15.7 v.s. 21.7±7.13 pg/ml) (P: ≤0.05). The study showed that the highest mean level of TNF-α was found in children after tooth extraction in the first week of after tooth extraction (33.2±9.1 pg/ml) and the level was still decreased to be the lowest level after one month tooth extraction of children (20.18±2.9 pg/ml), the result was significant (P: ≤0.05). The study showed that the highest mean level of IL-6 was found in children in the first week of after tooth extraction (92.1±15.8 pg/ml) and the level was still decreased to be the lowest level was after one month of tooth extraction (67.9±9.81 pg/ml), the result was significant (P: ≤0.05).

Keywords: TNF; IL-6; Tooth extraction; Inflammation.

Introduction

Current clinical practices show that health care providers and parents tend to underestimate children’s pain when compared with children’s self-reports¹. This incongruity results from the inability of young children to fully understand, verbalize, and express their experiences ². in conjunction with adults being unable to adequately detect and identify signs of pain in the pediatric population. Given that pain in children is inherently difficult to assess, pain may be unrecognized or undiagnosed resulting in a mistaken belief that infants and children suffer less than adults or do not feel pain ³. The International Association for the Study of Pain acknowledges that the “inability to communicate verbally does not negate the possibility that an individual is experiencing pain and (is) in need of appropriate pain management.” ¹. Dental extractions of primary teeth have been, and very often still are, carried out without any pain relief medication in the belief that children do not experience significant amounts of pain ⁴. Studies conducted to describe dentists’ knowledge of and attitudes towards procedural pain in children have revealed that dentists downplay procedural pain ⁵. Pain is likely the most significant morbidity associated with dental extractions. ⁶. However, for unknown reasons, the impact of these studies has been slow to permeate into clinical knowledge and translate into clinical intervention. Postoperative pain is often a new experience for young children. The complexity of interpreting and verbalizing pain may be convoluted further by unfamiliar postoperative sensations from general anesthesia (GA), surgical site discomfort, and disorientation. The recovery period after surgery may require formal assessments of pain in this population. Interleukin 6 (IL-6) is a proinflammatory cytokine, which is produced by a number of immune system cells; fibroblasts, macrophages, T and B Lymphocytes, endothelial cells, keratinocytes and tumor cells ⁷. Interleukin 6 (IL-6), as major mediator of the inflammatory response, plays a primary role in the inflammatory reaction ⁸. Based on these data, the inhibition of the IL-6/IL-6 receptor interaction with specific antibodies has been proposed as a support treatment of inflammation after tooth extraction ⁹. The cytokines interleukin-6 (IL-6), tumor
necrosis factor alpha (TNF alpha) and interleukin-1 beta (IL-1beta) are critical mediators of the inflammatory response\textsuperscript{10}. So the aim of the study was to estimate the level of TNF alpha and IL-6 in children after tooth extraction.

**Materials and Method**

A cross-sectional study was carried out in Kirkuk city from November 2018 to March 2019. The number of children after tooth extraction under study were 55 children ages were between 5-12 years old. These patients admitted to private clinics of dentists. The control group who were matched to the children with tooth extraction studied, included 35 healthy children. Three ml of blood was collected by vein puncture using Vacutainer tubes from each patient enrolled in this study for determination the level of IL-6 and TNF-α by using ELISA technique (Koma-biotech USA Co).

**Statistical Analysis**

Computerized statistically analysis was performed using Mintab ver 18.0 statistic program for determination of the $P$. value ($P <0.05$ considered significant).

**Finding**

The study showed that the highest mean level of TNF-α was found in children after tooth extraction comparing with healthy control group (36.8±13.4 v.s. 13.5±2.9 pg/ml) ($P: \leq 0.05$), Table 1.

**Table 1: Level of TNF-α beta in children after tooth extraction and the control group.**

<table>
<thead>
<tr>
<th>TNF-α level (pg/ml)</th>
<th>Patients</th>
<th>Control group</th>
<th>$P$. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>36.8</td>
<td>13.5</td>
<td>$\leq 0.05$</td>
</tr>
<tr>
<td>SD.</td>
<td>13.4</td>
<td>2.9</td>
<td></td>
</tr>
</tbody>
</table>

The highest mean level of IL-6 was found in children after tooth extraction comparing with control (88.1±15.7 v.s. 21.7±7.13 pg/ml) ($P: \leq 0.05$), Table 2.

**Table 2: Level of IL-6 beta in children after tooth extraction and the control group.**

<table>
<thead>
<tr>
<th>IL-6 (pg/ml)</th>
<th>Patients</th>
<th>Control group</th>
<th>$P$. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>88.1</td>
<td>21.7</td>
<td>$\leq 0.05$</td>
</tr>
<tr>
<td>SD.</td>
<td>15.7</td>
<td>7.13</td>
<td></td>
</tr>
</tbody>
</table>

The study showed that the highest mean level of TNF-α was found in children after tooth extraction in the first week of after tooth extraction (33.2±9.1 pg/ml) and the level was still decreased to be the lowest level after one month tooth extraction of children (20.18±2.9 pg/ml), the result was significant ($P: \leq 0.05$). The study showed that the highest mean level of IL-6 was found in children in the first week of after tooth extraction (92.1±15.8 pg/ml) and the level was still decreased to be the lowest level was after one month of tooth extraction (67.9±9.81 pg/ml), the result was significant ($P: \leq 0.05$), Table 3.

**Table 2: comparison among Level of TNF-α and IL-6 levels regarding the duration after tooth extraction**

<table>
<thead>
<tr>
<th>Interleukins levels (Mean±SD) pg/ml</th>
<th>First week</th>
<th>2 week</th>
<th>3 week</th>
<th>1 month</th>
<th>$P$. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>33.2±9.1</td>
<td>29.8±3.6</td>
<td>26.8±3.8</td>
<td>20.18±2.9</td>
<td>$\leq 0.05$</td>
</tr>
<tr>
<td>IL-6</td>
<td>92.1±15.8</td>
<td>80.6±13.2</td>
<td>77.8±10.8</td>
<td>67.9±9.81</td>
<td>$\leq 0.05$</td>
</tr>
</tbody>
</table>
Discussion

In this study, serum IL-6 level was evaluated in children after tooth extraction as compared to healthy controls. Interleukin-6 is found to be elevated in various inflammatory and malignant diseases including oral infection and their levels are found to correlate with the extent of the infection \(^9\)\(^-\)\(^1\(^1\)\). It is produced by some types of cancer cells and by normal stromal cells, such as fibroblasts and endothelial cells \(^5\)\(^-\)\(^6\). By acting as growth factor, IL-6 is able to promote tumor cell proliferation through upregulation of anti-apoptotic and angiogenic proteins in tumor cells. Also IL-6, is a major mediator of the inflammatory response, plays a primary role in the pathophysiology of wound infection \(^7\)\(^-\)\(^8\). The study results were in agreement with a recent study made by Rhodus et al \(^1\(^2\)\) carried out on 75 children after tooth extraction patients and 15 healthy controls. He found that serum IL-6 level for the patients was significantly higher than normal children and strongly correlated with disease progression. Also Yazid et al \(^1\(^3\)\) found that serum level of both IL-6 and IL-8 were found to be higher in patients than in healthy volunteers. Some other studies have mentioned that children after tooth extraction have more elevated levels of IL-6 and TNF alpha and their levels were negatively correlated with time after the process \((p < 0.01)\) \(^6\)-\(^8\). It was reported that IL-6 antitumor activity was enhanced by induction of induction of T cell and B cell differentiation, stimulation of cytotoxic T cells and help in producing lymphokineactivated killer cells \(^1\)\(^-\)\(^2\). We thought that This immune response trigger synthesis and release of this cytokine leading to augmentation of its serum level that might be utilized as a marker of immunity status and immune system activation in prognosis and monitoring of the course of infection \(^4\)\(^-\)\(^5\). This immune response might be attenuated with the progression of disease stage and inflammatory overwhelming which reflect decrease in (IL-6) synthesis \(^5\)-\(^6\).

Conclusions

There was a highly significant relation of TNF-α and IL-6 with children after tooth extraction and especially in first week after extraction of disease.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Kirkuk health Directorate and all experiments were carried out in accordance with approved guidelines.

References

11. A Motta, R Furini, J Simão, M Ferreira, M Komesu, N Foss, “The recurrence of leprosy reactional episodes could be associated with oral chronic infections and expression of serum IL-1, TNF-α, IL-6, IFN-γ and IL-10”, Brazilian dental journal, 2010; 21:158.
12. N Rhodus, B Cheng, S Myers, “A comparison of
the pro-inflammatory, NF-κB-dependent cytokines: TNF-alpha, IL-1-alpha, IL-6, and IL-8 in different oral fluids from oral lichen planus patients”, Clinical Immunology, 2005;114:278.

Effect of Psychoeducational Program on Eating Disorder Behaviors and Severity Level of Depression: A Quasi Experimental Study

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Abstract

Background: Major depressive disorder is one of the public health problems with prevalence among worldwide. Major Depression (MD) is the most prevalent comorbid mood disorder in eating disorder (ED) patients, and the severity of depressive symptomatology seems to be related to the ED. Most international clinical practice guidelines recommend psycho-educational intervention as first step in the treatment protocol. This study aimed to find out the effect of the psycho-educational program as the treatment of choice for depression patient with eating disorder.

Methods: A quantitative quasi experimental study with pre-post intervention design used to evaluate the effectiveness of psychoeducation program. A non-probability, purposive sample size of (50) patients with major depressive disorder were recruited to the study from January 10th 2018 till October 1st 2019. Beck depression inventory (BDI) was used for measuring the severity of depression, and Garner eating disorder inventory (G-EDI) was other measure used to assess eating behavior problems. Data of the present study was analyzed through using application of statistical package for social sciences (SPSS) version.

Results: The study indicated a significant difference in Beck depression score in intervention and control group after intervention (P< 0.001). It shows that the mean ranks of the difference (gain in scores) of the experimental group was significantly (p < 0.05) higher than the mean ranks of the difference of the control group for all the parameters of eating disorders.

Conclusion: The findings indicated that the 6-sessions psycho-education program has great impact on the severity of depression and all component of eating disorder in patient diagnosed with depression in associated with eating disorder.

Keywords: Major depressive disorder, eating disorders, psychoeducation program.

Introduction

Major depressive disorder is one of the public health problems, with prevalence 3.6% of global population. Major Depression (MD) is the most prevalent comorbid mood disorder in eating disorder (ED) patients, and the severity of depressive symptomatology seems to be related to the ED one. Lifetime comorbidity between eating disorders and mood disorders has been confirmed by several retrospective study, in anorexia nervosa the prevalence of mood disorders varies from 64.1% and 96% whereas in bulimia nervosa between 50% and more, and current comorbidity varies from 12.7 to 68% in anorexia nervosa and is about 40% in bulimia nervosa, and nearly half of BED patients have a comorbid mood.
It’s normal for people with eating disorders to feel depressed at some point, but most research shows 50-75% of those with eating disorders experience major depressive disorder, which can put them at greater risk for suicide. Depression seems to aggravate the severity of eating disorders. Thus, depression may be an important link between eating disorders and suicide. Most international clinical practice guidelines for the management of depression and feeding and eating disorders recommend psycho-educational intervention as first step in the treatment protocol. The psycho-education often precedes intensive forms of treatment or integrates with the other treatments. Recently, the National Institute for Health and Clinical Excellence (NICE) defined psycho-education as “Any structured group or individual program that addresses an illness from a multidimensional viewpoint including social, biological, familial, and pharmacological perspectives as well as providing service users and carers with information support and management strategies.” This study aimed to find out the effect of the psycho-educational program as the treatment of choice for depression patient with eating disorder.

Methodology

Study design:

A quantitative quasi experimental study with pre-post intervention design used to evaluate the effectiveness of psychoeducation program on eating behavior problems among outpatients with previously diagnosed with major depressive disorder. The study was carried out during the period from January 10th 2018 till October 1st 2019.

Study sample:

A non-probability, purposive sample size of (50) patients with major depressive disorder were recruited from attending Ali Kamal consultation center / psychiatric department in Sulaimani city for treatment and follow up. Patients being older than 18 years old and had drug adherence have been recruited to this study. Any patient was diagnosed with other mental disorders such as drug abuse, and pregnant women or puerperium were excluded from this study.

The study instrument:

The questionnaire includes socio-demographic characteristics such as patient’s age, gender, marital status, and level of education, employment status, residential area, income and BMI. And psychiatric characteristics: such as duration of depression, number of hospitalizations, suicidal attempts and family history of mental illness were part of the questionnaire.

Beck depression inventory (BDI) was used for measuring the severity of depression. It is a (21) multiple choice scale statements that evaluates key symptoms of depression including: feeling of sadness, pessimism, sense of failure, self-dissatisfaction, guilt, punishment, self-dislike, self-accusation, suicidal ideas, crying, irritability, social withdrawal, indecisiveness, body image change, work difficulty, insomnia, fatigability, loss of appetite, weight loss, somatic preoccupation and loss libido.

Measurement and scoring:

Beck depression inventory was 4 Likert scale in term of severity of the symptoms, it was scored from zero to 3. Each item of Beck depression inventory is ranked in terms of severity of the symptoms, and it was scored from absence of a symptom (zero) to an intense level (3). Severity of depression was based on the added scores of all items, and it was categorized to 5 levels, which was Normal range (1-10), Mild mood disturbances (11-16), Minimum depression (17-20), Mild depression (21-30), Moderate depression (31-40), and Severe depression (Over 40).

Eating disorder behaviors scale (G-EDI) is a list of (64) items related to (8) subscales, it is six Likert scale ranging from never to always. Items are scored as (never = 0, very rarely = 1, rarely = 2, occasionally = 3, frequently = 4, always = 5). For each sub-scale, item’s score was added, mean was calculated to find out the severity of the subscale. The higher the mean of score refers to the higher severity of eating disorder behaviors in each subscale.

Validity and reliability:

The face validity of the questionnaire was established through a panel of (13) experts of different specialists related to the field of the present study. The internal consistency of the instrument was determined through the computation of Cronbach’s Alpha test. the Cronbach Alpha test of the reliability of the questionnaire was (0.842).
**Statistical Method**

Data of the present study was analyzed through the application of statistical package for social sciences (SPSS) version (22). Mean and mean rank was calculated for each scale. Mann Whitney, Kruskal Wallis and Wilcoxon signed rank test were used to compare the mean ranks of two groups, test the significant relation of the study variables with scores. The P-value of ≤0.05 was considered statistically significant.

**Results and Discussion**

Table 1 indicated that there is a significant difference in Beck depression score in intervention and control after intervention (P<0.001). In the experimental group there had been marked improvements of the means of Garner scale scores after the intervention which is not true for the control group. It shows that the mean ranks of the difference (gain in scores) of the experimental group was significantly (p < 0.05) higher than the mean ranks of the difference of the control group for all the parameters of eating disorders.

Table (2) presents that the scores of gain in Garner scale after the intervention had been compared according to gender of the patient. The table indicates that the mean rank of difference for bulimia among females was 16.1 which was significantly higher than the mean rank (8.4) of the males (p = 0.009). The mean rank of difference of body dissatisfaction was 16.2 for females which was significantly higher than the mean rank of difference of males which was 8.2 (p = 0.008). On the other hand, regarding the ineffectiveness, the mean rank of difference of males (16.8) was significantly (p = 0.034) higher than that of females (10.5). No significant difference was detected between males and females in the mean ranks of the other subscales.

Regarding the employment status (Table 4), significant mean gains in Garner scale have been seen in drive for thinness, and body dissatisfaction. The mean ranks of drive for thinness of the self-employed and unemployed were significantly higher than those of those employed in governmental and private sectors (p = 0.038). Regarding the body dissatisfaction, the highest mean and mean rank of the difference was for the unemployed (p = 0.017).

Table (5) shows that there was no significant association between the mean ranks of the difference in scores of all eating behaviors and income except for the drive for thinness (p = 0.005), but it is evident here that there is no consistent trend.

In this study, the depression level was assessed through the Beck depression inventory (BDI). The mean and mean rank of the difference in Beck depression inventory scores before and after intervention show that the experimental group gained more points of improvement of depression symptoms (mean=23) than the control group (mean=4). The findings indicated large effect size within experimental group after post-intervention of 6-sessions psycho-education program while the effect size within control group was small over time of re-test assessment (P>0.001). The findings of this study showed that the mean and mean rank scores in the subscale bulimia and body dissatisfaction were significantly higher in females (8.2, 16.1) (14.3, 16.2) compare to males (2.0, 8.4) (4.4, 8.2) respectively, similarly with the results of 14, body dissatisfaction’s mean and mean ranks scores were significantly higher among unemployed subjects compared to other categories of employment status of intervention group. Additionally, the findings could be explained by the escape theory which suggests that some people narrow their level of attention to the current and immediate stimulus environment in order to distract attention from negative affect, for example depression, hence leading to external eating disorder behaviors 14.
Table 1. Means of the difference in Beck depression score before and after the intervention in the two study groups.

<table>
<thead>
<tr>
<th>Difference in Beck depression score (pre – post intervention) in the experimental group</th>
<th>Difference in Beck depression score (pre – post-test) in the control group</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>Mean rank</td>
<td>Mean</td>
</tr>
<tr>
<td>23.0</td>
<td>(38.0)</td>
<td>4.0</td>
</tr>
</tbody>
</table>

*By Mann-Whitney test

Table 2. Mean and mean rank of the difference between the pre and post intervention of eating disorder behaviors of the two study groups.

<table>
<thead>
<tr>
<th>Difference in scores of indicators†</th>
<th>Experimental group</th>
<th>Control group</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Mean rank</td>
<td>Mean</td>
</tr>
<tr>
<td>Drive for thinness</td>
<td>12.1</td>
<td>(35.94)</td>
<td>-1.2</td>
</tr>
<tr>
<td>Interpersonal distrust</td>
<td>10.4</td>
<td>(35.98)</td>
<td>2.0</td>
</tr>
<tr>
<td>Perfectionism</td>
<td>11.4</td>
<td>(37.88)</td>
<td>-0.5</td>
</tr>
<tr>
<td>Bulimia</td>
<td>5.7</td>
<td>(30.48)</td>
<td>0.8</td>
</tr>
<tr>
<td>Maturity fears</td>
<td>12.6</td>
<td>(37.62)</td>
<td>-3.5</td>
</tr>
<tr>
<td>Interoceptive awareness</td>
<td>11.5</td>
<td>(35.16)</td>
<td>4.0</td>
</tr>
<tr>
<td>Body dissatisfaction</td>
<td>10.3</td>
<td>(33.92)</td>
<td>0.4</td>
</tr>
<tr>
<td>Ineffectiveness</td>
<td>15.4</td>
<td>(35.56)</td>
<td>3.4</td>
</tr>
</tbody>
</table>

*By Mann Whitney test, comparing the mean ranks of the two groups.

†The difference in scores = reading before the intervention – reading after the intervention.

Table 3. Parameters of the difference (pre – post intervention) in eating disorder behaviors scores by gender in the intervention group.

<table>
<thead>
<tr>
<th>Eating disorders Indicators</th>
<th>Gender</th>
<th>Mean</th>
<th>Mean rank</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drive for thinness</td>
<td>Male</td>
<td>10.5</td>
<td>(10.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>13.2</td>
<td>(14.4)</td>
<td>0.242</td>
</tr>
<tr>
<td>Interpersonal distrust</td>
<td>Male</td>
<td>12.2</td>
<td>(16.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>9.3</td>
<td>(10.9)</td>
<td>0.078</td>
</tr>
<tr>
<td>Perfectionism</td>
<td>Male</td>
<td>12.5</td>
<td>(15.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>10.7</td>
<td>(11.1)</td>
<td>0.107</td>
</tr>
<tr>
<td>Bulimia</td>
<td>Male</td>
<td>2.0</td>
<td>(8.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>8.2</td>
<td>(16.1)</td>
<td>0.009</td>
</tr>
<tr>
<td>Maturity fears</td>
<td>Male</td>
<td>13.7</td>
<td>(15.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>11.8</td>
<td>(11.6)</td>
<td>0.242</td>
</tr>
</tbody>
</table>


Cont... Table 3. Parameters of the difference (pre – post intervention) in eating disorder behaviors scores by gender in the intervention group.

<table>
<thead>
<tr>
<th>Difference in scores</th>
<th>Employment</th>
<th>Mean</th>
<th>Mean rank</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drive for thinness</td>
<td>Employee public sector</td>
<td>4.0</td>
<td>(3.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Employee private sector</td>
<td>6.5</td>
<td>(6.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Self-employed</td>
<td>14.0</td>
<td>(15.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unemployed</td>
<td>13.8</td>
<td>(15.1)</td>
<td>0.038</td>
</tr>
<tr>
<td>Interpersonal distrust</td>
<td>Employee public sector</td>
<td>11.7</td>
<td>(15.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Employee private sector</td>
<td>8.0</td>
<td>(11.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Self-employed</td>
<td>12.1</td>
<td>(15.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unemployed</td>
<td>9.6</td>
<td>(11.3)</td>
<td>0.547</td>
</tr>
<tr>
<td>Perfectionism</td>
<td>Employee public sector</td>
<td>11.0</td>
<td>(11.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Employee private sector</td>
<td>12.5</td>
<td>(15.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Self-employed</td>
<td>12.4</td>
<td>(15.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unemployed</td>
<td>10.8</td>
<td>(11.7)</td>
<td>0.635</td>
</tr>
<tr>
<td>Bulimia</td>
<td>Employee public sector</td>
<td>6.0</td>
<td>(13.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Employee private sector</td>
<td>7.5</td>
<td>(14.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Self-employed</td>
<td>2.6</td>
<td>(9.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unemployed</td>
<td>7.1</td>
<td>(14.7)</td>
<td>0.485</td>
</tr>
<tr>
<td>Maturity fears</td>
<td>Employee public sector</td>
<td>14.0</td>
<td>(13.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Employee private sector</td>
<td>14.5</td>
<td>(17.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Self-employed</td>
<td>13.3</td>
<td>(15.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unemployed</td>
<td>11.5</td>
<td>(11.1)</td>
<td>0.521</td>
</tr>
</tbody>
</table>

*By Mann-Whitney test.

Table 4. Parameters of the difference (pre – post intervention) in eating disorders scores by occupation in the intervention group.
Table 4. Parameters of the difference (pre – post intervention) in eating disorders scores by occupation in the intervention group.

<table>
<thead>
<tr>
<th>Eating disorders</th>
<th>Income</th>
<th>Mean</th>
<th>Mean rank</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drive for thinness</td>
<td>Sufficient</td>
<td>18.0</td>
<td>(20.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Barely sufficient</td>
<td>6.7</td>
<td>(6.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Insufficient</td>
<td>14.5</td>
<td>(15.5)</td>
<td>0.005</td>
</tr>
<tr>
<td>Interpersonal distrust</td>
<td>Sufficient</td>
<td>12.3</td>
<td>(16.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Barely sufficient</td>
<td>8.0</td>
<td>(8.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Insufficient</td>
<td>11.7</td>
<td>(15.2)</td>
<td>0.069</td>
</tr>
<tr>
<td>Perfectionism</td>
<td>Sufficient</td>
<td>9.7</td>
<td>(7.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Barely sufficient</td>
<td>12.2</td>
<td>(14.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Insufficient</td>
<td>11.3</td>
<td>(13.1)</td>
<td>0.302</td>
</tr>
<tr>
<td>Bulimia</td>
<td>Sufficient</td>
<td>7.0</td>
<td>(16.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Barely sufficient</td>
<td>7.1</td>
<td>(14.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Insufficient</td>
<td>4.5</td>
<td>(11.2)</td>
<td>0.391</td>
</tr>
<tr>
<td>Maturity fears</td>
<td>Sufficient</td>
<td>11.7</td>
<td>(11.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Barely sufficient</td>
<td>12.4</td>
<td>(12.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Insufficient</td>
<td>12.8</td>
<td>(13.5)</td>
<td>0.877</td>
</tr>
<tr>
<td>Interoceptive awareness</td>
<td>Sufficient</td>
<td>10.7</td>
<td>(10.7)</td>
<td></td>
</tr>
</tbody>
</table>

*By Kruskal Wallis test.

Table 5. Parameters of the difference (pre– post intervention) in eating disorder behaviors scores by income in the intervention group.

<table>
<thead>
<tr>
<th>Eating disorders</th>
<th>Income</th>
<th>Mean</th>
<th>Mean rank</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drive for thinness</td>
<td>Sufficient</td>
<td>18.0</td>
<td>(20.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Barely sufficient</td>
<td>6.7</td>
<td>(6.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Insufficient</td>
<td>14.5</td>
<td>(15.5)</td>
<td>0.005</td>
</tr>
<tr>
<td>Interpersonal distrust</td>
<td>Sufficient</td>
<td>12.3</td>
<td>(16.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Barely sufficient</td>
<td>8.0</td>
<td>(8.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Insufficient</td>
<td>11.7</td>
<td>(15.2)</td>
<td>0.069</td>
</tr>
<tr>
<td>Perfectionism</td>
<td>Sufficient</td>
<td>9.7</td>
<td>(7.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Barely sufficient</td>
<td>12.2</td>
<td>(14.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Insufficient</td>
<td>11.3</td>
<td>(13.1)</td>
<td>0.302</td>
</tr>
<tr>
<td>Bulimia</td>
<td>Sufficient</td>
<td>7.0</td>
<td>(16.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Barely sufficient</td>
<td>7.1</td>
<td>(14.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Insufficient</td>
<td>4.5</td>
<td>(11.2)</td>
<td>0.391</td>
</tr>
<tr>
<td>Maturity fears</td>
<td>Sufficient</td>
<td>11.7</td>
<td>(11.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Barely sufficient</td>
<td>12.4</td>
<td>(12.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Insufficient</td>
<td>12.8</td>
<td>(13.5)</td>
<td>0.877</td>
</tr>
<tr>
<td>Interoceptive awareness</td>
<td>Sufficient</td>
<td>10.7</td>
<td>(10.7)</td>
<td></td>
</tr>
</tbody>
</table>
Conclusion

The findings indicated that the 6-sessions psycho-education program has great impact on the severity of depression and all component of eating disorder in patient diagnosed with depression in associated with eating disorder. The variance in the effect of this program had seen with difference socioeconomic status.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Family and Community Nursing Department and all experiments were carried out in accordance with approved guidelines.

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Cont...

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Effect of High Trace Element Levels in Serum of Women Undergoing Intra-cytoplasmic Sperm Injection (ICSI) on Implantation Rate

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1Assist. Lecturer, Ministry of Education, Baghdad, Iraq, 2Prof. Dr., Department of biology, College of Science -Al-Mustansiriya University, Baghdad, Iraq, 3Ph.D. Kamal AL-Samarrai hospital/Baghdad, Iraq

Abstract

Objectives: To assess the effects of trace element concentrations in serum on implantation rate in women undergoing ICSI.

Study design: Across-sectional study was conducted between March 2018 to April 2019 in Kamal AL-Samarai Hospital, center of fertility and IVF. One hundred and seventeen women underwent ICSI using GnRH-antagonist protocol were recruited. Concentration E2, P4 and two trace elements (Cu, Zn, and Cu/Zn) were measured in serum specimen. Women's were evaluated in two groups; the study group consisted of patients with successful implantation (n = 21) and women in the second group (n=96) experienced implantation failure.

Results: No significant differences were observed in age or BMI between the groups. Serum copper levels were significantly higher differences (P=0.001) in group of failure (198 ± 107μg/dL) than in group of successful implantation (133 ± 46μg/dL), zinc concentrations in group of successful and failure out of the maximum of normal range (137 ± 40μg/dL versus 135 ± 34μg/ dL respectively) but statistically significant differences were did not observed (P˃0.05). When we calculated the copper/zinc ratio (Cu/Zn), it was significantly higher differences (P=0.001) in group of failure (1.59 ± 1) than in group of successful (1.03 ± 0.4). In conclusion, we observed that high serum copper concentrations and (high Cu/Zn ratio) are a risk factor for implantation failure.


Introduction

The latest proposed definition of implantation failure is defined as a failure to achieve a clinical pregnancy after transfer of 4 or more good-quality embryos in a minimum of 2 in vitro fertilization (IVF) cycles in a woman under the age of 40 1, and caused by inadequate uterine receptivity in two-thirds of cases, and by problems with the embryo in the other third and it is a cause of infertility 2. Cobalt (Co), chromium (Cr), copper (Cu), manganese (Mn), molybdenum (Mo), and zinc (Zn) are essential for normal physiologic function and play significant roles in human and overall mammalian reproduction 3. However, few human data are available to describe the role of essential trace elements in IVF/ICSI, and their impact remains inconclusive.

Zinc is a trace element acts an antioxidant functions through Cu/Zn superoxide dismutase (SOD) and essential in vital functions such as cellular division and differentiation, making it essential for successful embryogenesis 4. It has been estimated that the total amount of zinc retained during pregnancy is ~100 mg 5. Because zinc is transported across the placenta via active transport from the mother to the fetus. Studies have shown that the fetus has notably higher zinc concentrations compared to the mother, even in cases of preeclampsia 6, indicating that the fetus, itself, can maintain adequate zinc homeostasis. Alteration in zinc homeostasis may have devastating effects on pregnancy outcome, including prolonged labor, fetal growth restriction, or embryonic and fetal death 7. On the other hand, copper is a redox-active transition metal and can
participate in single electron reactions and catalyze the formation of free radicals, including undesirable hydroxyl radicals; it could contribute to oxidative stress characteristic of preeclampsia. This illustrates that copper itself appears to be a pro-oxidant, but functions as an antioxidant when associated with Cu / Zn SOD. Cu/Zn SOD known to be expressed in both maternal and fetal tissues. Copper-containing contraceptive devices have been suggested to deposit copper ions in the endometrium, resulting in implantation failure. These findings led to the hypothesis that infertile patients with high serum copper concentrations may have experiences implantation failure due to the excessive accumulation of copper ions. Therefore, the aim of the present study was to investigate whether serum copper and Cu/Zn ratio concentrations are related to the implantation failure of human embryos in women undergoing intra-cytoplasmic injection.

Materials and Method

Subject selection

A cross-sectional study was conducted in a Center of Infertility Diagnosis and Assisted Reproductive Technology / Kamal AL-Samarai Hospital,(between March 2018 and April 2019). Women (n=117) were included in the present study. Patients aged between 20-45 years old with BMI > 29 kg/m² and duration of non-reproductive (2-24 years), out of these, 21 women who were successful implantation and 96 women who were implantation failure were compared. All patients provided written informed consent for the study, because our study involved the use of human data. Patients with gynecological disease such as endometriosis, endometrial polyps, fibroid in uterus and diabetes mellitus were excluded.

Ovarian stimulation and ICSI procedure.

Most of patients underwent ICSI treatment with fresh oocytes and freshly ejaculated spermatozoa. In a controlled-ovarian stimulation, rFSH (Gonal-F; Serono; Switzerland or Menogon; Ferring, GmbH Company; Germany) was injected intramuscularly from 2 nd day of menstruation to 2 days before oocyte retrieval, the dose was adjusted according to each individual case. A GnRH antagonist cetrorelix at 0.25 mg (Cetrotide®, Serono, Switzerland) or orgalutron (Organon company, The Netherlands) was administered when at least one follicle reached >14 mm. When more than two follicles were >18 mm, ovulation was triggered with a subcutaneous injection of recombinant human chorionic gonadotrophin (HCG) (Ovitrelle at 250 μg) (Merck- Serono, Geneva: Switzerland), it was administered 24–28 h after the final FSH administration. Vaginal ultrasound-guided aspiration of oocyte–cumulus complexes was performed 36 h later.

Oocyte denudation and ICSI were performed 3 hours after retrieval, and the in vitro culture was carried out in cleavage Gain medium (Fertipro/Belgium) under mineral oil until day 2 (2–5 cells stage) in automated incubators with 6% CO₂ at 37 °C, the growth of all the embryos from each patient (n=117) was continuously monitored. Embryo quality was assessed before embryo-transfer, and a maximum of three embryos transferred to all patients. Pregnancies were diagnosed by serum positive B-HCG levels (>100 mIU/ml) 14 days after embryo transfer.

Determination of parameters

Ethnicity was Iraqi only. Age, duration of non-reproductive, body mass index (BMI), endometrial thickness, E2 and P4, were assessed as possible confounders. Serum E2 and P4 were assessed using a solid-phase, competitive, enzyme immunoassay (Biomerieux/ France). Copper and zinc concentrations were measured in the day of embryo transfer by a clinical laboratory company (LTA/Italia). The trace elements were determined after digestion with chromogen. Briefly one volume of serum (50 μl) was mixed with two volumes of working reagent and for 5 min and then determined by atomic absorption spectrophotometer (Apel/Japan).

Statistical Analyses

Statistical analysis carried out by using Vassar Stats Web Site for Statistical Computation (Lowry, 2013). Measurable data expressed as (M ± SE). The Student’s t test (non-homogeneity, two-sided) was used for comparisons between two groups. The significance of differences estimated at two-tail P level less than 0.05

Results

Table 1 shows the demographic features and clinical parameters of the two groups. No significant differences (P>0.05) were observed in age, BMI, duration of non-reproductive, endometrial thickness, E2, and P4 between two study groups. Copper concentrations (mean ± standard deviation) were significantly higher
(P=0.001) in group of failure (198 ± 107μg/dL) than in group of successful implantation (133 ± 46μg/dL), while zinc concentrations of successful and failure out of the maximum of normal range (137 ± 40μg/dL versus 135 ± 34μg/dL respectively), but statistically significant differences were did not observed. When we calculated the copper/zinc ratio (Cu/Zn), it was significantly higher (P=0.001) in group of failure (1.59 ± 1) than in group of successful (1.03 ± 0.4) as shown in Table 2.

### Table 1 Demographic features and clinical parameters between two groups.

<table>
<thead>
<tr>
<th>Character (M±SD)</th>
<th>Implantation Group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Successful (n=21)</td>
<td>Failure (n=96)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>29.7 ± 1</td>
<td>31.4 ± 0.6</td>
</tr>
<tr>
<td>Infertility duration (years)</td>
<td>8 ± 1.1</td>
<td>7.7 ± 0.4</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>29.2 ± 3.3</td>
<td>28.4 ± 3.6</td>
</tr>
<tr>
<td>Endometrium thickness (mm)</td>
<td>9.3 ± 1.4</td>
<td>8.4 ± 1.2</td>
</tr>
<tr>
<td>E2(pg/ml)</td>
<td>1610 ±1003</td>
<td>1503 ± 914</td>
</tr>
<tr>
<td>P4(ng/ml)</td>
<td>35.4 ± 21.4</td>
<td>48 ± 37.2</td>
</tr>
</tbody>
</table>

Values indicate the mean ± standard deviation. BMI-body mass index, E2-Estridol, P4- Progesterone.

### Table 2 Comparison of copper and zinc levels in the serum of women with successful and failure implantation.

<table>
<thead>
<tr>
<th>Serum level (M±SD)</th>
<th>Normal range</th>
<th>Implantation Group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Successful (n=21)</td>
<td>Failure (n=96)</td>
<td></td>
</tr>
<tr>
<td>Copper (μg/dl)</td>
<td>80-155</td>
<td>133 ± 46</td>
<td>198 ± 107</td>
</tr>
<tr>
<td>Zinc (μg/dl)</td>
<td>70-115</td>
<td>137 ± 40</td>
<td>135 ± 34</td>
</tr>
<tr>
<td>Copper: Zinc ratio (Cu/Zn)</td>
<td>…..</td>
<td>1.03 ± 0.4</td>
<td>1.59 ± 1</td>
</tr>
</tbody>
</table>

**Discussion**

Our results suggest, for the first time, that high serum copper (low zinc) concentrations are related to implantation failure in Iraqi women.

In the present study, we found that group with failure implantation had significantly higher Cu concentrations and Cu/Zn ratios than groups of successful these results are consistent with previous studies by Tolunay and coworkers when were found decreased ongoing pregnancy chance with increasing follicular fluid of Cu concentrations. Prior research describes associations between essential trace elements in seminal plasma and semen quality parameters among infertile couples, and so is also likely to impact IVF outcomes. Copper, it is essential trace element, has a role in hemoglobin synthesis and immune function and is a cofactor for Cu/Zn superoxide dismutase (Cu/Zn-SOD) and ceruloplasmin. Copper and zinc are important in restoring or maintaining the oxidant–antioxidant balance in blood and tissues also can provide protection of cells against oxidative stress caused by ROS, which would
lead to damage of DNA or other important structures such as proteins and cell membranes. Studies indicated that an elevated Cu/Zn ratio was associated with increased oxidative stress, and oxidative stress closely associated with inflammation status, and the maintenance of redox balance is known to modulate immune system homeostasis. Chronic inflammation, as indicated by increased level of serum ceruloplasmin, is related to elevated level of C-reactive protein (CRP) and Cu. Changes in plasma levels of Cu and Zn has also been demonstrated for certain diseases, but an imbalance of the Cu/Zn ratio seems to be a better indicator of infection, vascular complications, several cancers including in the uterine endometrium, the pathogenesis of preeclampsia, and prognosis of diseases than Zn or Cu status alone. In addition, a previous study indicated that the Cu/Zn ratio may be a useful inflammatory–nutritional biomarker and predictor of mortality in elderly people. Regarding Zn, studies found higher blood Zn was associated with lower fecund ability in a prospective study of women trying to conceive, though of borderline statistical significance. Moreover, previous investigation reported that Zn deficiency increases the absorption of intestinal Cu and that Cu significantly inhibits the influx of Zn across the intestinal brush border membrane. At the molecular level, Zn increases the expression of Zn-finger protein A20, which can inhibit interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α), cytokines associated with inflammation.

Importantly, copper and zinc superoxide dismutase transcripts are present in human and mouse oocytes at germinal vesicle and metaphase II stages. The authors concluded that antral follicles might be more susceptible to Cu overexposure and undergo atresia or produce more corpora lutea as a result of ovulation. Other possible mechanisms for follicular damage include induction of cell apoptosis, vacuolization of the cytoplasm, and detachment of cell membrane from its cell membrane.

Again, Zn and Cu are well-known micronutrients that are important for the function of immune cells and the secretion of cytokines. In addition, higher levels of Cu can significantly decrease the number of circulating neutrophils, antibody titer, CD4/CD8 ratio, and NK cell activity.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Ministry of Education and all experiments were carried out in accordance with approved guidelines.

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Effect of Copper Nanopartical and Magnetized Salty Water in Chlorophylls and Carotenoids content of Tomato 
(*Lycopersicon esculentum* L.)

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1M.Sc. Student., Teacher in Al-janaeen High School, Ministry of Education, Babylon- Iraq, 2Prof. Dr., Department of Biology, College of Science, University of Babylon, Babylon –Iraq

ABSTRACT

The results of study showed that Cu nanoparticale at concentration of 5 M caused decreasing in chl.a content of tomato leaves significantly and the treatment of river water with Cu nanopartical at 5 M caused the highest value of chl.a (0.494 mg/g) , Cu nanoparticles of all it concentration caused decreasing in chl.b content of tomato leaves significantly comparing with control also with treatment of magnetic distilled water at 2000 Gauss, the concentrations of almost treatments increased the content of chl.b significantly comparing with control ,the treatment of drainage water(7 mmohs/cm) with magnetic water of 2000 Gauss caused the highest value of chl.b ( 0.695 mg/g). Cu nanoparticale at concentration of 0.5 M with magnetized distilled water caused decreasing in carotenoids of tomato leaves significantly, drainage water (7 mmohs/cm) with Cu nanoparticle at (0.5 and 9 M) caused decreasing in carotenoid comparing with control. The highest value of Cu nanopartical (9M) with drainage water ( 7 mmohs/cm) caused enhancement in carotenoid content (0.157 mg/g) comparing with control and treatment of drainage water at 7 mmohs/cm alone. Cu nanoparticale at concentration of 5 M caused decreasing of total chlorophyll content of tomato leaves significantly.

Key wards : Salinity, Cu NPs, Magnetic water, Lycopersicon esculntum L.

Introduction

A saline soil have a high concentration of soluble salts, high enough to affect plant growth. Salt stress is one of the major abiotic threats to plant life and significantly reduces crop yield in affected areas. Excessive salt above what plants need limits plant growth and productivity and can lead to plant death. About 20% of all irrigated land is affected by soil salinity, decreasing crop yields1. Salinity posses two major threats to plant growth: osmotic stress and ionic stress 2. Several studies have been conducted to investigate the salinity effect in plant growth and productivity 3. Exposed the castor (*Ricinus communis*) plant to salt stress caused a decrease in surface of leaf chlorophyll content, photosynthesis rate , and reduced plant productivity, noting that these effects increased with increasing salt concentration. It was also found that salt stress limits the growth of cucumber plant (*Cucumis sativus*) and causes dry of leaves 4. 5 had resulted that salinity cause a decrease in dry weight and water content of the rest of the *Vicia faba*. The results of the study 6 showed that the amount of chlorophylls (Chl.a , Chl.b ,Chl.a/b) and carotenoid varied by increasing or decreasing the period of exposure to salt stress in Ricinus plant. Some researcher (7,8) emphasized the increase in chlorophyll plant content in low salinity and its degradation with high salinity.

Nanoparticles are minutes finites with lengths ranging from 1-100 nanometers. They have unique physical and chemical properties. They have a large surface area to their size, making them highly motivating on influencing the growth and development of different types of plants, these effects may be either positive or negative 9.

Copper is one of the essential nutrients for plant growth in low concentrations because it needs very small quantities. Copper is involved in many vital processes to form protein and is the main component in the synthesis of many plant enzymes that activate oxygen reduction reactions such as cytochrome oxides, ascorbic acid oxides and lactase 10. Copper is widely distributed in plant tissues and is essential micronutrient for growth and involved in many physiological processes (11, 12).
It is widely used in agricultural industries, cosmetics, coatings, environmental remediation, fungicides, food industry, chemical industry, textile industries, medical industry, paints, plastics, wastewater treatment, and electronics. Copper as an element converts toxic above a threshold level, which depends on the type of crop plants. The plant content of copper ranges from 2 to 20 ppm in plant dry matter and has the highest concentration of copper in chloroplasts, also it plays an important role in the transmission chain of electrons and contributes to chlorophyll synthesis.

The magnetic field is defined as the magnetic force that arises in the area surrounding the magnetic body or in other words can be described as the area surrounded the magnet and shows the effect (in a given material) the magnetization of matter under the influence of an external magnetic field is due to the alignment of atoms or molecules of matter when the material exposed to the magnetic field becomes dipole of its atoms and its molecules are aligned towards the field used. Water is the most important factor for plant growth, the attempts to increase food and energy production for satisfying growing needs led to intensive development of plant production through the use of chemical additives, which in its turn caused more pollution of soil, water and air. Magnetic treatment of water has been reported to change some of the physical and chemical properties of water, mainly hydrogen bonding, polarity, surface tension, conductivity, pH and solubility of salts. These changes in water properties may be capable of affecting the growth of plants. The seedlings in the greenhouse were grown at a temperature (25±), the seedlings were treated with salt water (0, 2.83, 4, 7 mmohs/cm) and magnetized water (0, 2000, 3000 Gauss) and Cu nanoparticles in concentrations (0, 0.5, 5, 9 M) and interaction experiments between the three factors were treated with salt water and magnetized water by watering, while the nanomaterial was sprayed. The experiment was completed in February 1, 2019, and the leaves were taken at the age of four months of plant, the leaves between the third and fifth of the top of plant taken to determine of chlorophylls a, b, total chlorophyll and carotenoids from fresh samples, the chlorophyll content was determined by dipping a specific weight of (0.25) g in 15 ml of acetone (85%), and the samples were kept in the dark at room temperature 25±2 ºC for a week. Chlorophyll was then estimated by method, and the carotenoids content was estimated by method.

Materials and Method

**Tomato (Lycopersicon esculentum L.)** seedlings class california with two month-old were planted at 2 December, 2018. These seedlings were transferred to plastic pots containing a mixed soil: batmos with a ratio of 1: 1, capacity of 1.5 kg and 144 pots (48 treatments and 3 replicates per treatment). The physical and chemical properties of soil were analyzed (table 1) in the laboratories of the Soil Department / College of Agriculture / Al-Qasim Green University.

<table>
<thead>
<tr>
<th>Physical and chemical properties of soil</th>
<th>Sand</th>
<th>Silt</th>
<th>Clay</th>
<th>Ph</th>
<th>Ec</th>
<th>Ca</th>
<th>Mg</th>
<th>Na</th>
<th>K</th>
<th>Cl</th>
<th>SO4</th>
<th>CO3</th>
<th>HCO3</th>
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</thead>
<tbody>
<tr>
<td>gm /kgm</td>
<td>720</td>
<td>179</td>
<td>101</td>
<td>7.45</td>
<td>1.32</td>
<td>4.60</td>
<td>2.81</td>
<td>3.39</td>
<td>0.60</td>
<td>6.92</td>
<td>3.21</td>
<td>Nill</td>
<td>2.13</td>
</tr>
</tbody>
</table>

Table 1: Physical and chemical properties of soil
Results:

Table 2 refers that Cu nanoparticles at concentration of 5 M caused decreasing in chl. a content of tomato leaves significantly. All concentrations of all treatments increased the content of chl.a significantly comparing with control. The treatment of river water (2.83 mmohs/cm) with Cu nanoparticles of 5 M caused the highest value of chl.a (0.494 mg/g).

Table 2: Effect of copper nanoparticle and magnetized salty water in chlorophyll a mg/g f.w. of tomato (Lycopersicon esculentum L.)

<table>
<thead>
<tr>
<th>Salt concentration mmohs/cm</th>
<th>Cu nanoparticle M</th>
<th>Magnetic water Gauss</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td>d. W 0</td>
<td>0.049</td>
<td>0.135</td>
</tr>
<tr>
<td>2000</td>
<td>0.148</td>
<td>0.142</td>
</tr>
<tr>
<td>3000</td>
<td>0.249</td>
<td>0.333</td>
</tr>
<tr>
<td>River water 2.83</td>
<td>0.346</td>
<td>0.090</td>
</tr>
<tr>
<td>2000</td>
<td>0.126</td>
<td>0.270</td>
</tr>
<tr>
<td>3000</td>
<td>0.260</td>
<td>0.190</td>
</tr>
<tr>
<td>Drainage water 4</td>
<td>0.326</td>
<td>0.139</td>
</tr>
<tr>
<td>2000</td>
<td>0.225</td>
<td>0.180</td>
</tr>
<tr>
<td>3000</td>
<td>0.362</td>
<td>0.176</td>
</tr>
<tr>
<td>Drainage water 7</td>
<td>0.320</td>
<td>0.139</td>
</tr>
<tr>
<td>2000</td>
<td>0.279</td>
<td>0.153</td>
</tr>
<tr>
<td>3000</td>
<td>0.362</td>
<td>0.176</td>
</tr>
</tbody>
</table>

L.S.D. 0.05 = 0.006

Table 3 refers that Cu nanoparticles of all it concentration caused decreasing in chl.b content of tomato leaves significantly comparing with control also with treatment of magnetic distilled water at 2000 Gauss. The concentrations of almost treatments increased the content of chl.b significantly comparing with control. The treatment of drainage water (7 mmohs/cm) with magnetic water of 2000 Gauss caused the highest value of chl.b (0.695 mg/g).
Table 3: Effect of copper nanoparticle and magnetized salty water in chlorophyll b mg/g f.w. of tomato (*Lycopersicon esculentum* L.)

<table>
<thead>
<tr>
<th>Salt concentration mmohs/cm</th>
<th>Cu nanoparticle M Magnetic Water Gauss</th>
<th>0</th>
<th>0.5</th>
<th>5</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>d. W</td>
<td></td>
<td>0.072</td>
<td>0.036</td>
<td>0.060</td>
<td>0.041</td>
</tr>
<tr>
<td>2000</td>
<td></td>
<td>0.020</td>
<td>0.027</td>
<td>0.063</td>
<td>0.078</td>
</tr>
<tr>
<td>3000</td>
<td></td>
<td>0.148</td>
<td>0.086</td>
<td>0.191</td>
<td>0.147</td>
</tr>
<tr>
<td>River water 2.83</td>
<td></td>
<td>0.027</td>
<td>0.246</td>
<td>0.068</td>
<td>0.265</td>
</tr>
<tr>
<td>2000</td>
<td></td>
<td>0.386</td>
<td>0.126</td>
<td>0.245</td>
<td>0.308</td>
</tr>
<tr>
<td>3000</td>
<td></td>
<td>0.097</td>
<td>0.126</td>
<td>0.096</td>
<td>0.402</td>
</tr>
<tr>
<td>Drainage water 4</td>
<td></td>
<td>0.083</td>
<td>0.211</td>
<td>0.202</td>
<td>0.102</td>
</tr>
<tr>
<td>2000</td>
<td></td>
<td>0.262</td>
<td>0.592</td>
<td>0.075</td>
<td>0.051</td>
</tr>
<tr>
<td>3000</td>
<td></td>
<td>0.069</td>
<td>0.114</td>
<td>0.157</td>
<td>0.156</td>
</tr>
<tr>
<td>Drainage water 7</td>
<td></td>
<td>0.089</td>
<td>0.070</td>
<td>0.451</td>
<td>0.153</td>
</tr>
<tr>
<td>2000</td>
<td></td>
<td>0.695</td>
<td>0.212</td>
<td>0.176</td>
<td>0.247</td>
</tr>
<tr>
<td>3000</td>
<td></td>
<td>0.285</td>
<td>0.202</td>
<td>0.342</td>
<td>0.284</td>
</tr>
</tbody>
</table>

L.S.D(0.05) = 0.006

Table 4 indicate that Cu nanoparticle at concentration of 0.5 M with magnetized distilled water caused decreasing in carotinoids of tomato leaves significantly. Drainage water (7 mmohs/cm) with Cu nanoparticle at (0.5 and 9 M) caused decreasing in carotenoid comparing with control. The highest value of Cu nanoparticle (9M) with drainage water (7 mmohs/cm) caused enhancement in carotenoid content (0.157 mg/g) comparing with control and treatment of drainage water at 7 mmohs/cm alone.
Table 4: Effect of copper nanoparticle and magnetized salty water in carotinoids of tomato (*Lycopersicon esculentum* L.)*

<table>
<thead>
<tr>
<th>Salt concentration mmohs/cm</th>
<th>Cu nanoparticle M Magnetic water Gauss</th>
<th>0</th>
<th>0.5</th>
<th>5</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>d.W 0</td>
<td>0</td>
<td>0.080</td>
<td>0.099</td>
<td>0.083</td>
<td>0.087</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>0.075</td>
<td>0.072</td>
<td>0.077</td>
<td>0.092</td>
</tr>
<tr>
<td></td>
<td>3000</td>
<td>0.134</td>
<td>0.155</td>
<td>0.107</td>
<td>0.100</td>
</tr>
<tr>
<td>River water 2.83</td>
<td>0</td>
<td>0.150</td>
<td>0.110</td>
<td>0.119</td>
<td>0.094</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>0.134</td>
<td>0.080</td>
<td>0.084</td>
<td>0.128</td>
</tr>
<tr>
<td></td>
<td>3000</td>
<td>0.105</td>
<td>0.177</td>
<td>0.118</td>
<td>0.108</td>
</tr>
<tr>
<td>Drainage water 4</td>
<td>0</td>
<td>0.200</td>
<td>0.179</td>
<td>0.161</td>
<td>0.165</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>0.194</td>
<td>0.120</td>
<td>0.121</td>
<td>0.153</td>
</tr>
<tr>
<td></td>
<td>3000</td>
<td>0.083</td>
<td>0.089</td>
<td>0.101</td>
<td>0.168</td>
</tr>
<tr>
<td>Drainage water 7</td>
<td>0</td>
<td>0.129</td>
<td>0.013</td>
<td>0.183</td>
<td>0.072</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>0.105</td>
<td>0.126</td>
<td>0.202</td>
<td>0.193</td>
</tr>
<tr>
<td></td>
<td>3000</td>
<td>0.197</td>
<td>0.120</td>
<td>0.110</td>
<td>0.157</td>
</tr>
</tbody>
</table>

L.S.D 0.05 = 0.002

Table 5 refers that Cu nanoparticle at concentration of 5 M caused decreasing of total chlorophyll content of tomato leaves significantly. All concentrations of all treatments increased the content of total chlorophyll significantly comparing with control. The treatment of drainage water(7 mmohs/cm) with magnetic water of 2000 Gauss caused the highest value of total chlorophyll (0.978 mg/g).

Table 5: Effect of copper nanoparticle and magnetized salty water in total chlorophyll (mg/g f.w.) of tomato (*Lycopersicon esculentum* L.)

<table>
<thead>
<tr>
<th>Salt concentration mmohs/cm</th>
<th>Cu nanoparticle M Magnetic water Gauss</th>
<th>0</th>
<th>0.5</th>
<th>5</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>d.W 0</td>
<td>0</td>
<td>0.123</td>
<td>0.176</td>
<td>0.099</td>
<td>0.158</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>0.173</td>
<td>0.174</td>
<td>0.256</td>
<td>0.377</td>
</tr>
<tr>
<td></td>
<td>3000</td>
<td>0.403</td>
<td>0.424</td>
<td>0.649</td>
<td>0.547</td>
</tr>
<tr>
<td>River water 2.83</td>
<td>0</td>
<td>0.334</td>
<td>0.339</td>
<td>0.551</td>
<td>0.654</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>0.518</td>
<td>0.591</td>
<td>0.512</td>
<td>0.399</td>
</tr>
<tr>
<td></td>
<td>3000</td>
<td>0.330</td>
<td>0.293</td>
<td>0.449</td>
<td>0.786</td>
</tr>
</tbody>
</table>
Cont... Table 5: Effect of copper nanoparticle and magnetized salty water in total chlorophyll (mg/g f.w.) of tomato (*Lycopersicon esculentum* L.)

<table>
<thead>
<tr>
<th>drainage water 4</th>
<th>0</th>
<th>0.427</th>
<th>0.653</th>
<th>0.447</th>
<th>0.476</th>
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</thead>
<tbody>
<tr>
<td>2000</td>
<td>0.527</td>
<td>0.794</td>
<td>0.457</td>
<td>0.384</td>
<td></td>
</tr>
<tr>
<td>3000</td>
<td>0.297</td>
<td>0.335</td>
<td>0.295</td>
<td>0.441</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>drainage water 7</th>
<th>0</th>
<th>0.412</th>
<th>0.212</th>
<th>0.842</th>
<th>0.422</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>0.978</td>
<td>0.282</td>
<td>0.571</td>
<td>0.577</td>
<td></td>
</tr>
<tr>
<td>3000</td>
<td>0.765</td>
<td>0.457</td>
<td>0.728</td>
<td>0.710</td>
<td></td>
</tr>
</tbody>
</table>

L.S.D.0.05 = 0.008

**Discussion**

The plant growth subjected to high levels of Cu nanoparticle leads to reduce in biomass, chlorotic in leaves as well as decreasing in chlorophyll content, which is resulted from the change in the chloroplast structure lead to increase oxidation fat and then reduces the content of fatty acids. The Cu nanoparticle appears like the normal copper effect, nanoparticles cause many changes morphological and physiological to plants depending on the characteristics of these nanoparticles, may vary the efficiency of nanoparticles by the chemical composition, size and space surface and reactivity as well as the dependent on the type of plant and concentration as it is different from plant to time

**Conclusion**

1- The river water (2.83 mmohs/cm) and drainage water (4 and 7 mmohs/cm) enhanced the chlorophyll content.

2- The copper nanoparticles at different concentrations vary in effects on chlorophylls but these were no change in carotenoids significantly.

3- All the type water in current study which magnetized of 2000 Gauss enhanced the chlorophylls and carotenoid.

4- The interaction between types of water, Cu nanoparticles and magnetized water, all of increased the chlorophyll content comparing with control.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Ministry of Education, Iraq and all experiments were carried out in accordance with approved guidelines.

**References**

8. Salama S, Trivedi S, Busheva M. Effects of NaCl salinity on growth, cation accumulation, chloroplast...


Effectiveness of an Educational Program on Physically-Disabled Fighters’ Quality of Life at Rehabilitation Military Center in Al-Basra City

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² Prof. Dr., Community Health Nursing Department, College of Nursing, University of Baghdad, Iraq

Abstract

Objective(s): The aim of the study is to determine the effectiveness of the education program on physically-disabled fighters’ quality of life, to determine the effectiveness of the education program on physically-disabled fighters’ health status.

Methods: A quasi-experimental, one group test retest design, is carried out in order to achieve the objectives of the study by using the evaluation approach and the implementation of the education program at the Rehabilitation Military Center in Al-Basra City from of January 7th, 2019 to October 31st 2019. Non Probability “purposive” sample of (27) physically disabled fighters’ is selected for the present study. The sample is assigned according to the following criteria: a. Physically disabled who has not participated in any health educational program related quality of life. b. Physically disabled fighters are selected as having disability ratio from 60 to 80 percent. Test-retest reliability and content validity “panel of (18) experts” of the questionnaire are determined through pilot study.

Data are collected through the application of the education program, the use of the questionnaire and the structured interview as means of data collection. Data were analyzed through the use of descriptive statistical data analysis approach of frequency, percentage, mean, mean of scores, total of scores, range and standard deviation and inferential statistical data analysis approach T-test, multiple linear regressions, person correlation coefficient, Chi-Square test, and analysis of variance (ANOVA).

Results: The results of the study depict that the evaluation of physically-disabled fighters’ health status and quality of life is moderate due to the effect of physical disability; the physically disabled fighters’ quality of life domains have been improved due to the effect of the education program; the physically disabled fighters’ health status have been improved due to the effect of the education program; physically disabled fighters’ quality of life is significantly different relative to their age, residency, marital status, level of education, and chronic illness, and a non-significant difference relative to their type of injury and socioeconomic is determined.

Conclusion: The study concluded that the health education Program has produced an effect on the physically-disabled fighters’ health status in the study group post its implementation. So, the study has confirmed that the program is an effective mean for the physically-disabled fighters’ health status in the Rehabilitation Military Center; the Health Education Program has produced an effect on the physically-disabled fighters’ quality of life in the study group post its implementation. Further, the study has confirmed that the program is an effective mean for the enhancement of the physically-disabled fighters’ quality of life in the Rehabilitation Military Center.

Keyword: Educational Program, Quality of Life, Physically-Disabled Fighters’.
**Introduction**

The World Health Organization (WHO) defines a disability as something that impairs, limits activity, or constrains an individual’s participation.

Physical disability is defined as “the loss of motor function of varying degrees or limitation in movements or activities resulting from deformed limbs, body paralysis, or deformity caused by damage to the structure or function of body parts.”

A physical disability is the long-term loss or impairment of part of a person’s body function, resulting in a limitation of physical functioning, mobility, dexterity or stamina. Due to the functional loss the person will experience the inability to perform normal movements of the body, such as walking and mobility, sitting and standing, use of hands and arms, muscle control.

A Physical disability, which are also referred to as orthopedic, neuromotor, or musculoskeletal impairments, are those that prevent normal physical functioning. Examples of physical disabilities include cerebral palsy, a neuromotor disorder resulting from brain injury that impairs movement control, and spinal bifida, a neuromotor impairment of the spinal cord that can limit normal movement and cognitive functioning.

Quality of life is described as a wellness resulting from a combination of physical, functional, emotional and social factors. It is a broad multidimensional concept that includes subjective evaluations of both positive and negative aspects of life. Physical disability can decrease quality of life and limit the community participation. A significant and negative association was found between disability and quality of life.

The disabling nature of the chronic disease can limit the daily activities, restrict the society roles, unemployment and finally lead to drop the quality of life, the quality of life can be affected by disability.

People with physical disability are more likely to have chronic diseases such as cardiovascular disease, diabetes, stroke, arthritis, asthma, high blood pressure, cholesterol, and exposure to bed ulcers. The most common health problems among the physically disabled are obesity, which limits the freedom of movement and work in the environment, and limits the ability of the disabled to work daily activities such as feeding, bathing, and thus limit the ability of the person to rely on him-self.

Physically disabled people experience more restrictions in social activities than healthy people, which are associated with lower level of wellbeing.

The education is effects on people’s earnings and productivity; education helps them in achieving a variety of outcomes that matter for quality of life. Education is one of the most important predictors of one’s health status, Employability and probability of being married, all are well known predictors of quality of life.

Health education can improve quality of life, emotional function and lessen distress. Education improves well-being because it increases access to non-alienated paid work and economic resources that increase the sense of control over life, as well as access to stable social relationships, especially marriage, that increases social support.

Patient education significantly improves quality of life. It is concluded that patient education has a reliable positive impact on the quality of life. Education influences positively health condition and by this quality of life. The educational process passes also knowledge, skills and psycho-emotional motivations, allowing partner interaction between a patient and a teacher. The aims of education are though making patients independent, producing motivation for therapy and counteract fear, depression and frustrations.

**Method**

A quasi-experimental, one group test retest design, is carried out in order to achieve the objectives of the study by using the evaluation approach and the implementation of the educational program at the Rehabilitation Military Center in Al-Basra City from of January 7th, 2019 to October 31st 2019. The present study is conducted on physically disabled fighters at the Rehabilitation Military Center in Al-Basra City. The Military Rehabilitation Center is established on May 7th, 2018. It provides medical and rehabilitative services to physically disabled fighters’ who have disability ratio from 60 to 80 percent. Non Probability “purposive” sample of (27) physically disabled fighters’ has been selected for the present study. The physically disabled group is exposed to the educational program. The sample is assigned according to the following criteria:

a. Physically disabled who has not participated in any health educational program related quality of life.

b. Physically disabled fighters are selected as having disability ratio from 60 to 80 percent.
An educational program is constructed for the purpose of the study. The program is oriented toward the physically-disabled fighters’ quality of life. The program is comprised of (6) lectures. These lectures focus on physically-disabled fighters’ quality of life related issues.

The questionnaire is composed of three main parts as follows:

**Part I:** The Physically Disabled Socio-Demographic Characteristics: This part includes items of age, gender, residency, types of injury, marital status, level of education, chronic diseases, and socioeconomic status which are calculated through use for Socioeconomic Status Scale Low= (less than 59), Moderate= (60-80), and High= (81-100).

**Part II:** The Physically Disabled Fighters’ Health Status Evaluation Tool: This part is comprised of (32) item that measure physically disabled fighters’ health status about physically disabled fighters’. It is measured as High= (2.34-3) it mean poor health status, Moderate= (1.67-2.33) it mean moderate health status, and Low= (1-1.66) that means good health status.

**Part III:** Physically Disabled Fighters’ Quality of Life Evaluation Tool: This part is comprised of (69) item that measure physically disabled fighters’ quality of life about physically disabled fighters’. It is measured as High= (2.34-3), Moderate= (1.67-2.33), and Low= (1-1.66).

Test-retest reliability is employed for the determination of the study instruments stability. Pearson correlation coefficient is computed on responses of (12) physically disabled fighters at the Rehabilitation Military Center in Al-Basra City, determined through a pilot study. The content validity of the program and the study tools are determined through the use of panel of (18) experts.

Data are collected through the application of the education program, the use of the study instruments and the structured interview technique as means of data collection.

The data of the present study are analysed through the use of the Statistical Package of Social Sciences (SPSS) version 20. Through descriptive statistics (frequency, percentage, mean, mean of scores, total of scores, range and standard deviation) and statistical inferential (T-test, multiple linear regressions, person correlation coefficient, Chi-Square test, and analysis of variance ANOVA).

Results were determined as highly significant at (P≤0.01) significant at (P≤0.05) and non-significant at (P>0.05).

### Table (1): Comparative Differences among the three Periods (Pre-test, Post-test I and Post-test II) for the Physically-disabled Fighters’ Health Status

<table>
<thead>
<tr>
<th>Main Domain Related to Physically-Disabled Fighters’ Health Status</th>
<th>ANOVA</th>
<th>Test</th>
<th>Sum of Squares</th>
<th>d.f.</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Physical Health</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td></td>
<td>17.124</td>
<td>2</td>
<td>8.562</td>
<td>186.345</td>
<td>0.000</td>
<td>HS</td>
</tr>
<tr>
<td>Within Groups</td>
<td></td>
<td>3.584</td>
<td>78</td>
<td>0.046</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Mental and Psychological Health</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td></td>
<td>17.590</td>
<td>2</td>
<td>8.795</td>
<td>108.125</td>
<td>0.000</td>
<td>HS</td>
</tr>
<tr>
<td>Within Groups</td>
<td></td>
<td>6.345</td>
<td>78</td>
<td>0.081</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Social Health</strong></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td></td>
<td>18.142</td>
<td>2</td>
<td>9.071</td>
<td>97.548</td>
<td>0.000</td>
<td>HS</td>
</tr>
<tr>
<td>Within Groups</td>
<td></td>
<td>7.253</td>
<td>78</td>
<td>0.093</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Behavioral Health</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td></td>
<td>7.657</td>
<td>2</td>
<td>3.829</td>
<td>77.769</td>
<td>0.000</td>
<td>HS</td>
</tr>
<tr>
<td>Within Groups</td>
<td></td>
<td>3.840</td>
<td>78</td>
<td>0.049</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS: Non Significant at P ≥ 0.05, Sig: Significant at P < 0.05, HS: Highly Significant at P < 0.05, df: degree of freedom, F: F Observed.
This table revealed that a statistically high significant difference between the three period (pre-test, post-test I and post-test II) related to domain of physically disabled fighters’ health status, when analyzed by ANOVA.

**Table (2): Comparative Differences among the Three Period (Pre-test, Post-test I and Post-test II) for the Physically Disabled Fighters’ Quality of Life**

<table>
<thead>
<tr>
<th>Main Domain Related to Physically- Disabled Fighters’ Health Status</th>
<th>ANOVA</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test</td>
<td>Sum of Squares</td>
</tr>
<tr>
<td>Physical Health</td>
<td>Between Groups</td>
<td>5.833</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>2.853</td>
</tr>
<tr>
<td>Psychological Health</td>
<td>Between Groups</td>
<td>7.323</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>6.198</td>
</tr>
<tr>
<td>Social Relationship</td>
<td>Between Groups</td>
<td>1.817</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>4.112</td>
</tr>
<tr>
<td>Level of Independence</td>
<td>Between Groups</td>
<td>6.486</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>3.204</td>
</tr>
<tr>
<td>Environment</td>
<td>Between Groups</td>
<td>4.398</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>2.853</td>
</tr>
<tr>
<td>Spiritual Health</td>
<td>Between Groups</td>
<td>4.233</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>5.909</td>
</tr>
</tbody>
</table>

NS: Non Significant at P ≥ 0.05, Sig: Significant at P < 0.05, HS: Highly Significant at P < 0.05, df: degree of freedom, F: F Observed.

This table presented that a statistically high significant difference among the three period (pre-test, post-test I and post-test II) related to quality of life domains, when analyzed by ANOVA.
Table (3): Comparative Differences Between the Physically Disabled Fighters’ Quality of Life Relative to their (Ages, Residency, Types of injury, Marital Status, Level of Education, Chronic Disease, and Socio-economic Status)

<table>
<thead>
<tr>
<th>Quality of Life Variables</th>
<th>Pre-test Quality of Life</th>
<th>Post-test I Quality of Life</th>
<th>Post-test II Quality of Life</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chi-Square</td>
<td>d.f</td>
<td>p-value</td>
</tr>
<tr>
<td>Age (Years)</td>
<td>75.641</td>
<td>10</td>
<td>0.000</td>
</tr>
<tr>
<td>Residency</td>
<td>11.517</td>
<td>2</td>
<td>0.003</td>
</tr>
<tr>
<td>Types of injury</td>
<td>9.737</td>
<td>6</td>
<td>0.136</td>
</tr>
<tr>
<td>Marital Status</td>
<td>0.868</td>
<td>2</td>
<td>0.648</td>
</tr>
<tr>
<td>Level of Education</td>
<td>97.159</td>
<td>8</td>
<td>0.000</td>
</tr>
<tr>
<td>Chronic Disease</td>
<td>12.010</td>
<td>2</td>
<td>0.002</td>
</tr>
<tr>
<td>Socio-economic Status</td>
<td>1.683</td>
<td>4</td>
<td>0.794</td>
</tr>
</tbody>
</table>

This table revealed that there was a high significant relationship between physically disabled fighters’ quality of life and their age in pre-test for study group at (P < 0.05), while in post-test I shows that there was significant relationship between physically disabled fighters’ quality of life and their age at (P < 0.05), and there was a non-significant relationship between physically disabled fighters’ quality of life and their age in post-test II at (P ≥ 0.05). This table shows that there was a significant relationship between physically disabled fighters’ quality of life and their residence in pre-test for study group at (P < 0.05), while in post-test I and post-test II shows that there was non-significant relationship between physically disabled fighters’ quality of life and their residence in post-test II at (P ≥ 0.05). This table shows that there was a significant relationship between physically disabled fighters’ quality of life and their type of injury in pre-test for study group at (P < 0.05), while in post-test I and post-test II shows that there was non-significant relationship between physically disabled fighters’ quality of life and their type of injury at (P ≥ 0.05). This table shows that there was a non-significant relationship between physically disabled fighters’ quality of life and their marital status in pre-test for study group at (P ≥ 0.05), while in post-test I shows that there was a significant relationship between physically disabled fighters’ quality of life and their marital status at (P < 0.05), and there was non-significant relationship between physically disabled fighters’ quality of life and their marital status in post-test II at (P ≥ 0.05). This table shows that there was a significant relationship between physically disabled fighters’ quality of life and their level of education in pre-test for study group at (P < 0.05), and this table shows that non-significant different between physically disabled fighters’ quality of life and their level of education in post-test I and post-test II at (P ≥ 0.05). This table shows that there was a significant relationship between physically disabled fighters’ quality of life and their level of education in post-test II at (P ≥ 0.05).
relationship between physically disabled fighters’ quality of life and their chronic disease in pre-tests for study group at (P < 0.05), and this table shows that non-significant different between quality of life and chronic disease in post-test I and post-test II at (P ≥ 0.05). This table shows that there are no significant among three periods (pre-test, post-test I and post-test II) related to physically disabled fighters’ quality of life and their socio-economic status at (P ≥ 0.05).

Discussion

Part I: Discussion of the Effectiveness of the Education Program on Physically-Disabled Fighters’ Health Status

Analysis of such physically disabled fighters’ health status between the three period (pre-test, post-test I and post-test II) related to physically disabled fighters’ health status, in the present study, have moderate health status before the educational program implementation, and improved health status after educational program implementation (Table 1). This finding provides empirical evidence that these physically disabled fighters’ is perfect candidates to the education program because they experience health related problems and improved physically disabled fighters’ health status after implementation of educational program. Throughout the course of data analysis, the study depicts that physically disabled fighters’ health status has been improved particularly for those in the study group. So, we can confirm that physically disabled fighters’ in the study have benefited out of the education program implementation and the quality of life program can be considered an effective tool to improve physically-disabled fighters’ quality of life in the rehabilitation military center.

This finding is in an agreement with study conduct by Javed and others conduct a study to examine the effect of education on quality of life and well-being. The study depicts that an education influences people’s perception of their quality of life domains and well-being.

Part II: Discussion of the Effectiveness of the Education Program on Physically-Disabled Fighters’ Quality of Life and their Socio-demographic Characteristics

Analysis of such differences reveals that physically-disabled fighters’ quality of life is significant different relative to their age, residency, marital status, level of education, and chronic illness, except that of type of injury, and socioeconomic status which depicts not significant different relative physically disabled fighters’ quality of life (Tables 3).

This finding is in an agreement with that of a study indicate there is a statistically significant difference between quality of life and their: age, marital status, and educational level.

Conclusion

1. Evaluation of physically-disabled fighters’ health status and quality of life is moderate due to the effect of physical disability.
2. The physically disabled fighters’ quality of life domains have been enhanced due to the effect of the education program.

3. The physically disabled fighters’ health status has been improved due to the effect of the education program.

4. Physically disabled fighters’ quality of life and socio-demographic characteristics. Physically-disabled fighters’ quality of life is significant different relative to their age, residency, marital status, level of education, and chronic illness, and a non-significant difference relative to their type of injury and socioeconomic is determined.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Ministry of Defense, Iraq and all experiments were carried out in accordance with approved guidelines.

**References**


Antibacterial Activity and Phytochemical Screening of Iraqi Taraxacum Officinale L

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Abstract

Objective: The objective of this research is to identify the microscopic Characteristics in plant extraction by detecting effective component and examine of antibacterial vitality.

Procedure: Taraxacum officinale leaf was gathering of Baghdad city. After the prepare the leaf and the preparation and scrub and then dried we extracted by the SOXHLET device where we use 90% ethanol. This pharmacological study includes microscopy and a chemical screening using reagent. Through the diffusion method an antibacterial evaluation of the leaf extract was detected. in this treatise was used diverse bacteria Pseudomonas aeruginosa, Klebsiela pneumonia, Staphylococcus aureus, E. coli, Enterococcus faecalis and Streptococcus spp.

Outcomes: An outcome indicates the presence in the top and down surface of the leaf type of stomata is the stomatal actinocytic while non-glandular trichome is found only above the top surface of the leaf. As the outcome of microscopic screening of the forms of fiber that the indicators for the top area of the leaf was 18%, while the down region was 31% for the leaf. The Initial outcome of chemical plant screening for plant leaf was revealed existence at Tannins, Saponins, Terpenoids and Alkaloids component. The outcome of effectiveness against bacteria in the search was offered showed diverse activity against all bacterial species within search.

Keywords: Taraxacum officinale, stomata, phytochemical screening

Introduction

Dandelion is Perennial herbaceous plant belongs to the family Asteraceae (Compositae) Also called T. officinale, green areas and on the sides of the road, Banks, beaches and areas with moist soil. This plant has been used in herbal medical fields. It was also mentioned specifically in Asia, Europe and North America. For the roots of the plant is very useful for the treatment of problems of the gastrointestinal tract support liver function and digestion while plant leaves are used as diuretic and digestive stimulants. Medicinal plants contain a range of active compounds of great importance and therapeutic properties as most studies indicate the increasing activities of antimicrobial plants by detecting areas targeted by antibiotics by plant extracts against drug-resistant microbial pathogens. The curative Characteristics of plant extracts can be introduced into traditional or folk medicine by interacting with certain species and producing antioxidant activities. Dandelion plant has a protective effect against brain damage caused by lead. The root of the dandelion plant contains some substances that have antioxidant property these substances are inulins, lactones, triterpenes, sterols, flavonoids, and phenolic acids. Through studies in China and America found dandelion plant containing antiperspirants through its inhibition of NO production and cyclooxygenase-2 (COX-2) acts as anti-inflammatory and antinociceptive. In recent years it has increase important to find naturally occurring antioxidants for food safety for the purpose of promoting health and preventing various infections and their high degree of safety in human use and consumer acceptability.
Material and Method

Collection of Plant Material

A leaf of dandelion plant was aggregated of region saydiya in Iraq. After obtaining the leaf of the plant was wash out by distilled water and scour with strainer paper to remove powder and suspended parts and then drying temperature up to 25-30°C for one week and persistent stirring to block the formation of molds. It was then weigh, grind, placed in tight vials and stocked for use in the extraction process. The plant sample was identified and authenticated by national herbarium of ministry of agriculture of Iraq.

Extraction

The plant samples (leaves of plant) were washed dried under shade conditions for 10 days. After drying the leaves were powdered in a mechanical grinder. 300 g of leaves powder was packed in the thimble of Soxhlet apparatus and extracted with 900 ml of aqueous ethanol (ethanol-water 80:20 v/v) for 12 hr. extract was filtered and concentrated under vacuum using rotary evaporator to get a dry residue. 12 g of residue was used in screening the different active compounds.

Preliminary Phytochemical Investigation

Preliminary investigations for the chemical constituents were done using 5% ethanolic potassium hydroxide (KOH) for detect of flavonoids, mayers and dragendroffs reagents for detection of alkaloids and foam test for saponins, 1% lead acetate test, ferric chloride (FeCl3) and alkaline test for tannin.

Macroscopic Examination

Fresh specimens of plant were used to study the morphological characters of the plant such as a shape of leaves, stems and margins.

Microscopic Examination

Powdered Microscopy

Dried leaves were finely powered used for examined the anatomical study and observed the plant tissue component, teeny magnitude of the dust was putted in slide to clarify by few drizzle from the chloral hydrate and wrapped for covering skid for tested under microscope. Different cell components were observed and taken the figures by microscopical camera.

Leaf Microscopy

For determination the type of stomata and type of trichomes used two surface of leaves. The lower and upper epidermal layer of fresh leaf were clarifying by chloral hydrate and heating for many times and observed under a microscope. Determination of the components of leaves (stomata, trichomes and stomatal index) were carried out under microscope. The stomatal index was carried out by using the following equation.

\[
\text{Stomatal index} = \frac{\text{No: of Stomata}}{\text{No: of Stomata} + \text{No: of epidermal cells}} \times 100
\]

Antimicrobial examination

Efficiency is gauged through the essential disc sawing way. All pure culture from bacteria was possessed to the lab of microbiology, basic Science College of dentistry of Mustansiriyah University. Pseudomonas aeruginosa, Klebsiela pneumonia, Staphylococcus aureus, E. coli, Enterococcus faecalis and Streptococcus spp. It was cultivated in a culture medium where each was mixed with a concentration of Taraxacum officinale extract, Inhibition zone were determine through fisher-lilly zone. The concentricity of streptomycin was the measurement was made-up in Three copies.
Outcomes and debate

Macroscopical and Plant Morphological

Dandelion cultivate of Of single and uncomplicated roots, it grow more than approximately 10 stem 7–36 cm long, however occasionally up to 66 cm long. a outcome of the morphological study were referred to the leafs are 3–40 cm long and 2–8 cm broad. The leaf border is usually Lobular and often ruptured or serrated with severe teeth.

Microscopical Examination

Outcome was reference to actinocytic type of stomata was presented on top and down exterior of the leafs, whilst the non-glandular unicellular trichomes offered on top exterior of the leafs. Also the microscopic Outcome was reference to streak form fibers.

Phytochemical Screening

Outcomes from leaves extract were presented and referred to the extract was contain the Tannins, Saponins, Terpenoids and Alkaloids compounds.

Antimicrobial Activity

The plant anatomical results were reference to Anomocytic stomata type in the leaves. Unicellular unbranched covering of trichomes, fibers and starch present in the leaves of the Taraxcum officinale plant (figure 3,4,5,6). The phytochemical profile results of active constitutes were referenced to many active compounds were presented such as tannin, saponins, Tannins, Saponins, Terpenoids and Alkaloids which isolated and purificated Table (2). The pathogenic bacteria which used for investigated the bioactivity of leaves extract of Taraxcum officinale were E coli, pseudomonas aeruginosa, Streptococcus spp., Staphylococcus epidermidis, Enterococcus faecalis, Klebsiella pneumoniae and Staphylococcus aureus. The different concentrations of cardioactive glycosides extract from leaves (1000,500,250,125,63.5µg /ml) were found active for all microorganisms strain in concentration 1000 µg /ml while inactive in 63.5 µg /ml concentration exception Enterococcus faecalis effected in this concentration. The largest inhibition zone found in Enterococcus faecalis in all concentrations while the smallest inhibition zone found in Staphylococcus epidermidis bacteria. In obtained results shown that Taraxcum officinale extract effect for all types of bacteria that mean this extract has good potency against all tested bacteria seen in table (1) and figure (2).

**Table (1) Antibacterial activity of ethanolic extract of leaves of Taraxcum officinale**

<table>
<thead>
<tr>
<th>Pathogenic bacteria</th>
<th>inhibition zones (mm) , concentration (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1000</td>
</tr>
<tr>
<td>E coli</td>
<td>20</td>
</tr>
<tr>
<td>pseudomonas aeruginosa</td>
<td>18</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>23</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>19</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>13</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>27</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>11</td>
</tr>
</tbody>
</table>
Table (2) Phytochemical screening of Taraxicum officinale.

<table>
<thead>
<tr>
<th>Test</th>
<th>Roots</th>
<th>Leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Microscopical Examination

Figure (3): Anomocytic stomata Figure (4): Starch

Figure (5): Annual xylem vessels Figure (6): Fiber

Conclusion

According to the both results Pharmacognostical, phytochemical and antimicrobial study the expended of cultivation of this plant in Iraq is very necessary to improve quality and quantity and investigation the activity in animal study.

Financial Disclosure: There is no financial disclosure.
Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Mustansiriyah University and all experiments were carried out in accordance with approved guidelines.

References


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Effect of Different Resin Luting Materials on the Marginal Fit of Lithium Disilicate CAD/CAM Crowns
(A Comparative Study)

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Abstract

Aim of the study: To recognize the effect of using different resin luting materials on the vertical marginal discrepancy of Lithium Disilicate CAD/CAM crowns.

Material and method: A total of 36 intact maxillary first premolars extracted for orthodontic purpose were disinfected in a solution of 1% Thymol for 1 day which utilized to receive a ceramic crown after tooth preparation. Digital impression was made with Omnicam Scanner (Dentsply Sirona) using Cerec Premium Software. Lithium disilicate CAD/CAM crowns made via In-Lab MC XL milling device (Dentsply Sirona) and cemented with different resin luting materials. The marginal discrepancy was measured at three locations on each tooth surface, with optical microscope at 200x magnifications before and after cementation. Statistical analysis was performed with one way ANOVA test to know statistical significance and Turkey’s test (HSD) to compare the mean marginal increase among the three groups (P=0.05).

Statistical analysis: The least amount of marginal increase after cementation was with Gaenial universal flo (flowable composite); with a mean marginal increase of 38.53 ±0.63 mm. Choice2 cement (resin cement) increased the margins by mean 40.55 ±0.95 mm. The highest marginal increase was detected in the Ceramx one sphere TEC (preheated composite) resin group (87.82±1.26 mm).

Conclusion: Cementation of CAD/CAM crowns with preheated composite resin (Ceramx sphere TEC one) cause a marginal increase surpassed the clinically acceptable range of marginal discrepancy.

Keywords: resin cement, flowable, preheated, Lithium Disilicate, Marginal discrepancy, CAD/CAM.

Introduction

The vertical marginal discrepancy considered as an integral part of indirect restoration and this discrepancy increased after cementation and poor marginal fit can lead to microleakage, marginal discoloration, dissolution of cement and secondary caries. Type of cement used can govern the amount of augmentation in discrepancy after cementation, but to which degree computer aided design/ computer assisted manufacture (CAD/CAM) Lithium Disilicate crown affected by different resin cement is unclear. Marginal discrepancy is a perpendicular distances from the margin of a restoration to the-finish-line-of the tooth preparation¹. Although-horizontal discrepancies such as crown overhangs intraorally can be adjusted to some degree; but the vertical marginal-discrepancy can be closed only by the luting cement,-which-is-prone-to-degradation. Therefore, the-vertical marginal-discrepancy- has the-most-clinical significance-and-should-be considered-the more-critical-element in-crown margin-evaluation². Marginal fit considered as a basic factor in the success of indirect restoration. Poor marginal fit may cause cement dissolution which lead microleakage or secondary caries³. Therefore; to-decrease the-incidence-of above mentioned complications minimal marginal discrepancy is-an essential element for long term restoration success. Conflicting studies proposed regarding the clinical acceptable marginal-discrepancy. Some studies recommend a marginal discrepancy of less than 120 μm⁴ and others less than 100 μm⁵. Different brands of luting
cements have been used for the adhesive luting of ceramic crowns, and attitudes vary as to which is the best. Dual-polymerized resin cements have been designated when the ceramic material is too thick or too opaque to allow satisfactory polymerizing light transmission\(^6\). Furthermore, flowable composite resins have been advised for adhesive luting. Barcelheiro et al\(^9\) found similar results when bonding feldspathic porcelain to bovine enamel by using dual polymerized resin cement and a light polymerized flowable composite resin. Their study has been advised clinicians to use flowable composite resins as an appropriate substitute luting agent when bonding porcelain laminate veneers less than 2 mm in thickness.

In addition preheated composite resins have also been used to bond restorations. Preheating composite resin decreases its viscosity and ultimate film thickness, offering the clinician an improved controlling and superior composite resin adaptation to preparation margins, together with an increased degree of polymerization and depth of polymerization\(^10\).

There is no specific cementation protocol for lithium-disilicate restorations can be considered ideal\(^9\). Moreover, researches regarding the influence of luting cement on marginal discrepancy after cementation provide different results\(^10\).

This in vitro study to recognize the effect of using different resin luting materials on the vertical marginal discrepancy of Lithium Disilicate CAD/CAM crowns is proposed.

**Material and Method**

Thirty-six sound human maxillary first premolar teeth of equivalent size and shape extracted for orthodontic purpose from patients with age range 17-22 years had been selected to be used in this in vitro study. Cleaning of teeth has been done carefully from any calculus and soft tissue deposits with air Scaler then disinfected in a solution of 1% Thymol for 1 day.

To avoid dehydration of the specimens during all stages of the study samples has been stored in distilled water at room temperature\(^11\). All teeth samples has been implanted individually in cold cure acrylic resin block up to 2 mm apical to the CEJ to approximate the support of alveolar bone in a healthy tooth then the teeth prepared with high-speed turbine mounted on dental surveyor (Dentauro, Germany) (Fig. 1), with copious water spray under ×4 magnification (Carl Zeiss dental loupe) to receive a ceramic crown according to the following criteria: a planar occlusal surface reduction, 1.0 mm deep chamfer finishing line depth, 6 degree convergence angle and 5 mm height from the occlusal level to the intended finish line both buccally, and palatally (Fig. 2).

**Figure (1): high-speed turbine mounted on dental surveyor**

Direct scanning was done by powder-free Omnicam intraoral scanner (Dentsply Sirona Dental System, Bensheim, Germany) by using CEREC premium software (version 4.5), then In Lab MCXL (Dentsply Sirona Dental Systems, Bensheim, Germany) was used to produce the full ceramic crown using-CEREC in-Lab (version 18.0) software

**Figure (2): Finished prepared tooth**

A: Lateral view, B: Occlusal view

The crowns where divided into, three. Groups (twelve crowns in each group) according, to the luting cement to be used

A-flowable composite group (G-ænial Universal Flo, GC Japan)

B-light cure, resin cement, group (Choice 2 cement;
C-pre-heated composite, group (ceramx sphere TEC one, Dentsply, Germany)

The inner surface of the crowns for all groups has been treated similarly before cementation (table 1) also all teeth of all groups has been treated similarly (table 2). Measurements were performed with a dino-light digital microscope at magnification of 200X.

**Table 1. Treatment of internal surfaces of crowns**

<table>
<thead>
<tr>
<th></th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Etching with 9% hydrofluoric acid for 20 sec.</td>
</tr>
<tr>
<td>2</td>
<td>Water rinse for 20 sec. then air drying</td>
</tr>
<tr>
<td>3</td>
<td>Silane application with micro-brush for 20 sec. then air dry</td>
</tr>
</tbody>
</table>

The measurements were performed at three locations on each tooth surface therefore; a total of 12 marginal adaptation evaluation sites for each tooth were performed.

After that measurements performed before and after adhesive cementation and the difference between the above mentioned tow measurements will considered as a marginal discrepancy. Statistical analysis was performed with one way ANOVA test to know statistical significance and Tukey test (HSD) has been performed to compare the mean marginal increase among the three groups. (P=.05)

**Table 2. Treatment of teeth**

<table>
<thead>
<tr>
<th></th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Etching enamel with phosphoric acid 37% (Total etch) for 15 sec.</td>
</tr>
<tr>
<td>2</td>
<td>Rinsing with water for 15 sec. before air drying</td>
</tr>
<tr>
<td>3</td>
<td>Bond (universal bond, bisco, USA) on enamel and dentin with microbrush</td>
</tr>
<tr>
<td>4</td>
<td>Flowable composite resin (Gaenial universal flo, GC), resin cement (choice 2, bisco) application inside crown In case of preheated composite resin (ceramx sphere TEC one) Using Micerium heater (ENA heat, Micerium) for 55 min. at temperature 55oC then applied inside the crown (ENA)</td>
</tr>
<tr>
<td>5</td>
<td>Cleaning access with composite modeling brush</td>
</tr>
<tr>
<td>6</td>
<td>Light polymerization for 20 sec. for each surface</td>
</tr>
</tbody>
</table>

Results

Statistical analysis was performed with one way ANOVA test to know statistical significance and significant differences were found among the groups (P=.000) and tukey test (HSD) has been performed to compare the mean marginal increase among the three groups. The mean marginal increase after cementation for Gaenial univer-sal flo (flowable composite) was 38.53±0.63 mm. For Choice2 cement (resin cement) was 40.55±0.95 mm and for Ceramx sphere TEC one (preheated composite) group 87.82±1.26 mm (table 3). Representative digital image of the crowns before and after cementation are shown in Figure 3.

Discussion

The Results of this study has been revealed that significant differences among the groups (P=.000). The increase in the marginal discrepancy after adhesive luting of restorations was constant with that of other studies.

Outcomes of the present study demonstrated an increase in marginal discrepancies of 38.53±0.63 μm with flowable composite (Gaenial universal flo), 40.55±0.95 μm with resin cement (choice 2), and 87.82±1.26 μm with preheated composite (ceramx sphere TEC one).

Results have been demonstrated that preheated composite (ceramx sphere TEC) had a meaningfully higher value of marginal increase than flowable composite (Gaenial universal flo) and resin cement (choice 2). These results agree with those of the study by Sampaio et al who stated that preheated restorative composite resin (68_C) had a higher film thickness (300 μm) than flowable resins (150 μm). Results of the current study also agree with those findings by Blalock et al who acknowledged an average film thickness of flowable composite resin (35 μm) and preheated composite resin at 54_C (140 μm). The reported marginal increases after adhesive luting have ranged between 13 and 50 μm. Flowable composite (Gaenial universal flo) and resin cement (choice 2) used in this investigation seemed to meet the International Organization for Standardization requirement which is 50 μm maximum film thickness, because the increase in marginal gaps after cementation was within the recommended limit, where is preheated (ceramx sphere TEC) much higher than recommended and this agree with Stappert et al who found that 20 to 50 μm marginal increase for e.max restorations.
cemented with resin luting cement (Variolink II; Ivoclar Vivadent AG).

In 2013 Sakrana examined the fit of two types of ceramic single crowns and indirect composite resin complete coverage crowns before and after cementation with self-adhesive resin cement and found an increase in the marginal discrepancy after cementation ranging from 24 to 40 mm.

Results of the present study revealed that preheated composite (ceramx sphere TEC) resin caused a large marginal increase (87.82 ±1.26) which is above the required limit, placing results out of the range of marginal increase of previous studies. Therefore, it is not recommended for crown cementation; instead, flowable composite (Gaenial universal flo) are recommended beside the resin cement (choice 2).

The above mentioned facts agree with Mounajjed et al. who identified that the marginal increase of pressed lithium disilicate crowns cemented with preheated composite resin exceeded the clinically acceptable range of marginal discrepancy. Beside that manufacture literature stated that Gaenial Universal Flo represents a simple and efficient solution for the cementation of veneers. This versatile, injectable restorative composite unites easy handling, high physical properties and excellent aesthetics. Generally used as a restorative and liner material, it can also be used for the bonding of veneers and some inlays and onlays where light-curing is possible and considered as good alternative to preheated composite.

Some studies reported a mean increase of the margins of approximately 17 μm. Other study by Pascale et al. stated that luting of inlay, onlay and overlay with preheated restoration composite doesn’t prevent seating accuracy, these results disagree with our findings.

According to Shinkai et al., the rate of flow of luting agent depends largely on the amount and configuration of the filler particles. This is explain that why flowable composite (Gaenial universal flo) has highest flow as it contain 69 % fillers by weight compared to resin cement (choice 2) 75 % fillers by weight and preheated composite (ceramx sphere TEC) 77-79% fillers by weight and regarding to configuration Gaenial universal flo has an ultra-fine spherical particles (strontium glass) which tend to improve fracture strength and increase flow rate.

Masouras et al. stated that in addition to the amount, size, and type of filler material, the coupling of the resin matrix to the filler particles played an important role in the material’s performance, according to manufactured Gaenial universal flo has a new silane surface treatment which is called full coverage silane coating (FSC) technology which makes it possible to homogeneously and densely disperse ultrafine 200 nm fillers. Flexural strength considered as the most important physical properties of luting agent, according to manufactured Gaenial universal flo has very high flexural strength (167 MPa) compared to choice 2 resin cement (124 MPa) and ceramx sphere TEC (146 MPa) thanks to the new filler filler technology which allowed to develop an injectable material that is stronger than most conventional composite.

On the topic of the manufactured, one of exclusive advantages of Gaenial universal flo its viscosity which is carefully balanced in order to provide material that has a thixotropic property which provides easy placement and flow smoothly under pressure.

According to the above mentioned properties and findings, Gaenial universal flo can be considered as a suitable alternative luting agent when bonding lithium disilicate crowns that were less than two millimeter in thickness.

Conclusions

Within the limitations of this in vitro study, the following conclusions:

1. The cementation process increased the marginal discrepancy for the 3 luting cements evaluated.
2. Preheated composite resin (ceramx sphere TEC one) had produced significantly higher marginal discrepancies than flowable composite resin (Gaenial universal flo) or resin cement (Choice 2).

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Dentistry and all experiments were carried out in accordance with approved guidelines.
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Antibacterial and Antifungal Activities of Centaureabruguierana (Asteraceae) from Iraq

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Abstract

The methanol and aqueous extracts of Centaureabruguierana. (Asteraceae) from Iraq were tested for their biological activity against two Gram positive, two Gram negative bacteria and four fungal species by using agar well diffusion method. The crude methanol extract (100mg/ml) and its dilutions and the crude aqueous extract( 100mg/ml) showed biological activity against all bacteria and fungi studied. Compared with the aqueous extracts, methanol extracts, particularly the crude one, were more bioactive against bacteria and fungi studied. Among the studied microbes, E.coli, Staphylococcus aureus and Microsporum canis were the most sensitive species to C.bruguierana extracts tested. Methanol extract at all concentrations and aqueous extract at high concentrations showed close or more antibacterial activity( except in case of P.auruginosa) than that of the bacterial control ( tetracycline) . The crude methanol extract was more bioactive against all fungi studied than the fungal control ( nystatin ).This is the first report on the antibacterial and antifungal activities of C.bruguierana extracts from Iraq and its bordering countries.

Kew words : Antibacterial, Antifungal, Asteraceae, Centaurea, Iraq.

Introduction

Centaurea is the fourth largest genus in the Asteraceae family, including 300 -700 species[1][2][3][4] with annual, biennial and perennial plants [5] that distributed all around the world especially in Mediterranean sea region and west Asia [1][4]. In Iraq, this genus was represented by more than 30 species [6]. Species of this genus have been used in folk medicine as diuretic , stomachic , tonic, astringent, antimalarial ,antirheumatic, antidandruff, hypoglycemic and antipyretic [2][7][8][9][10]. Many investigations have been indicated the presence of different classes of active compounds in this genus which in many cases are responsible for its biological activity[10][11]. C. bruguierana (DC.)Hand.-Mazz. was reported for the first time from Iraq [12]. Our previous study [13] showed a high flavonoid content in this species. However, there is no reports on the antimicrobial activity of C. bruguierana in the literature .Therefore, this study was conducted to evaluate the effects of the methanol and aqueous extracts of this species in four bacterial species ( two Gram positive and two Gram negative ) and four fungal species .

Materials and Method

Plant materials and extraction method

C.bruguierana samples were collected from Tikrit province/ Salahadin Governorate (north central Iraq) in March 2018 . Plant samples were identified according [14] and confirmed by the Iraqi National Herbarium. Identified samples were kept in the Department of Biology, College of Education for Pure Sciences,TikritUniversity,Iraq . Aerial flowering parts of C.bruguierana were oven dried at 40ºC and then grinded with electrical mill. 40 g of the powdered samples were extracted with 160ml of 95%methanol or hot distilled water for 24 hours, then the liquid was filtered through gauze colth and filter paper Whatman No.1 and the filtrate was concentrated at 40ºC. The solid residue was stored in the fridge until subsequent use . For in vitro assay ,1gm of the solid
residue were dissolved in 10ml of the same solvents (methanol or distilled water) and tested at concentrations 25,50,100mg/ml. These concentrations were evaluated in triplicate for bacteria or fungi tested. Before use, the crude extracts (100mg/ml) were filtered by using Millipore filter with Whatman filter paper No. 0.22 [15][16].

**Microorganisms and antimicrobial assay**

Two Gram positive( *Staphylococcus aureus*, *Bacillus pumilus*) and two Gram negative( *Escherichia coli*, *Pseudomonas aeruginosa*) bacterial species, three fungal species(*Microsporumcanis*, *Trichophytonrubrum*, *Asperrillus terreus*) and one yeast (*Candida albicans*)were used in this study. All bacteria and molds were obtained from the Biology Department, College of Science, Baghdad University and the yeast was supplied from the Department of Biology, College of Science, Tikrit University. Nutritive media used included nutrient agar and MacConkey agar for bacteria and Sabouraud dextrose agar and potato dextrose agar for fungi(all media from Bekasi, Indonesia).抗菌和抗真菌活动被用经过好扩散方法[16][17]。对于抗菌和抗真菌测试,tetracycline和nystatin(100µg/ml for each) were used as positive control while methanol and sterilized distilled water were used as negative control.

**Results and Discussion**

The biological activities of the methanol and aqueous extracts of *C. bruguierana* against bacterial and fungal species tested in this study are presented in Table 1; Fig.s.1,2. The results showed that all C. *bruguierana* extracts exhibited inhibitory activity against all bacterial species studied while these extracts showed some activity on fungi tested. Methanol extracts revealed strong activity against both bacterial and fungal species studied compared with the aqueous extracts. The activity of the studied extracts was directly proportional with their concentrations. As shown in Table 1, *S.aureus*, *E.coli* and *M.canis* were the most sensitive to the extracts tested especially at high concentrations. In case of bacteria(Table 1 and Fig.1), methanol extract of *C. bruguierana* at all concentrations showed inhibition zone diameters (16.1-25mm) very close or greater than the standard antibiotic tetracycline (16.4mm) . Methanol extract only at high concentration (100mg/ml) gave inhibition zones diameter (17-20mm) greater than the antifungal nystatin (15mm). Aqueous extracts except few cases (*B. pumilus* and *P. aeruginosa*) showed inhibition zones close or greater (15.1-25.4mm) than the control etracycline (Table 1 and Fig.2). Aqueous extracts at all concentrations showed lower inhibition zones diameter than the antifungal nystatin. Table 1 revealed that the mean inhibition zone diameters exhibited by the methanol extracts(19.1-23.6mm for bacteria and 14.2-16.7mm for fungi) were greater than those exhibited by the aqueous extracts (3.0-22.6mm). The high flavonoid contents previously reported in *C. bruguierana* [13] may be responsible for the antimicrobial activity of this species [10][11]. According to the literatures, this is the first report on the antimicrobial activity of the *C. bruguierana*. The present study was in agreement with the previous studies on the antimicrobial activity of the other *Centaurea* species, particularly their methanol extracts [3][6][7][8][10][18][19]. However, further studies are needed to evaluate the biological activity of methanol extracts of *C. bruguierana* against other pathogenic bacteria and fungi because their antimicrobial activities are more than that of the of the standard antibiotic tetracycline and antimycoticnystatin.

**Conclusion**

This is the first study on the antimicrobial activities of *C. bruguierana* extracts. This study showed a promising antimicrobial activities of *C. bruguierana* extracts, particularly methanol extracts. Methanol extracts exhibited stronger antibacterial and antifungal activities than the standard antibiotic tetracycline and the antifungal nystatin. This plant species could be a source for antimicrobial drug against pathogenic microorganisms, particularly multiresistant bacteria.
### Table 1. Antibacterial and antifungal activities of the methanol extract from C. bruguierana

<table>
<thead>
<tr>
<th>Microbes</th>
<th>methanol extract (mg/ml)</th>
<th>aqueous extract (mg/ml)</th>
<th>mean methanol extract</th>
<th>mean aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>25</td>
<td>50</td>
<td>100</td>
<td>25</td>
</tr>
<tr>
<td>S. aureus</td>
<td>20.9</td>
<td>24.9</td>
<td>25.0</td>
<td>20.0</td>
</tr>
<tr>
<td>B. pumilus</td>
<td>16.1</td>
<td>19.2</td>
<td>22.6</td>
<td>13.2</td>
</tr>
<tr>
<td>E. coli</td>
<td>020.</td>
<td>23.2</td>
<td>25.9</td>
<td>20.1</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>18.3</td>
<td>19.2</td>
<td>20.9</td>
<td>9.0</td>
</tr>
<tr>
<td>C. albicans</td>
<td>10.3</td>
<td>10.5</td>
<td>019.</td>
<td>0.0</td>
</tr>
<tr>
<td>T. rubrum</td>
<td>14.0</td>
<td>17.0</td>
<td>19.0</td>
<td>0.0</td>
</tr>
<tr>
<td>M. canis</td>
<td>14.0</td>
<td>14.0</td>
<td>20.0</td>
<td>9.0</td>
</tr>
<tr>
<td>A. terreus</td>
<td>14.0</td>
<td>16.0</td>
<td>17.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Tetacycline</td>
<td>16.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nystatin</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methanol</td>
<td>0.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>0.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 1.** Antimicrobial activity of the methanol extracts of C. bruguierana.  
St: standard
Conflict of interest: There is no conflict of interest among the authors.

Funding: Self

Ethical Clearance: This study is ethically approved by the Institutional ethical Committee.

References


12. Al-Abassi S.H.: Active constituents and antimicrobial activity of some Centaurea species from central


Colorimetric Determination of Uric Acid in Live samples

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2Assistant Professor, 3Lecture, Department of Chemistry, College of Science, Mustansiriyah University,
Baghdad, Iraq

Abstract

A simple, sensitive and precise colorimetric method for uric acid determination was described. The method based on the complexion reaction between uric acid, potassium ferricyanide, and ferric chloride in hydrochloric acid medium to form blue colored charge transfer complex measured at maximum wavelength 752 nm. The optimum conditions obtained were volume 0.3 ml for hydrochloric acid, 1.25 ml for both potassium ferricyanide and ferric chloride solutions, 60°C and 8 minutes as maximum temperature and time of reaction, the order of mixing additives was uric acid, ferric chloride, potassium ferricyanide, and hydrochloric acid. The highly accurate and precise results obtained with RSD%, Recovery%, Ere%, and D.L were (0.755 to 4.376), (99.676 to 97.831), (-0.033 to -2.169), and 0.1569 µg mL⁻¹ respectively, the calibration graphs were linear in the concentration range of (2.0-200µg ml⁻¹), the molar absorptivity (5937.473L mol⁻¹ cm⁻¹) The proposed methods were successfully applied for the determination of uric acid in serum and urine samples for twenty persons.

Keywords: Uric acid, colorimetric, determination, charge transfer complex.

Introduction

Uric acid is a heterocyclic compound of carbon, nitrogen, oxygen, and hydrogen with the formula C₅H₄N₄O₃. It forms ions and salts known as urates and acid urates, such as ammonium acid urate. Systematic IUPAC name is 7,9-Dihydro-1H-purine-2,6,8(3H)-trione(1).

Uric acid is an end product of the breakdown of compound called purine. Purines (adenine and guanine) are chemical compounds plays a critical role in many body biological processes, by producing some important bioactive molecules; these molecules have a major part in genetic materials synthesis, protein synthesis, energy molecules production, and can act as mediators in nervous system(2). The major sources of purines are from diet, body synthesis from non-purine precursors, and turnover of nucleic acid(3).

Many analytical techniques were used for the determination of uric acid including spectrophotometric(4-9), fluromatric(10-13), enzymatic(14, 15), chromatographic(16-19), potentiometric(20-22), amperometric(23-27), and voltammetric (28-30) techniques but these techniques required sophisticated instruments and expensive reagents. Hence, the development of charge transfer complex based on the reaction between uric acid, potassium ferricyanide, and ferric chloride in hydrochloric acid medium was the aim of this study due to its simplicity, accuracy and economy of this method.

Experimental

Apparatus

UV-Visible double beam spectrophotometer (JASCO V-650, Japan), Sensitive balance ± 0.0001 g (Sartorius BL 210 S Scientific balance, Gottingen–Germany), Hot plat (Labtech, Germany), water path (Lab tech, Korea) and centrifuge (Kokusan, Japan) were required in this study.

Reagents and chemical materials

All chemicals used were of analytical reagent...
grade including [uric acid \((C_5H_4N_4O_3)\) sigma aldrich], potassium ferricyanide\(K_3[Fe(CN)_6]\), purity 99%, BDH), ferric chloride \((FeCl_3\), purity 96.8%, BDH) and hydrochloric acid \((HCl, 37\text{%, Carolina})\).

**Preparation of solutions**

Uric acid stock solution \((1000 \mu\text{mole L}^{-1})\):- One hundred milligram of uric acid standard material was dissolved by distilled water in a 100 ml volumetric flask.

Potassium ferricyanide solution \((0.001 \text{mole L}^{-1})\):- 0.326 gram of potassium ferricyanide standard material was dissolved in distilled water in 100 ml volumetric flask, 10 ml of this solution was diluted with 100ml distilled water to produce \((0.001\text{mole L}^{-1})\).

Ferric chloride solution \((0.002 \text{mole L}^{-1})\):- 0.162 gram of Ferric chloride standard material was dissolved in distilled water in 100 ml volumetric flask, 20ml of this solution diluted with 100ml distilled water to produce \((0.002\text{mole L}^{-1})\).

Hydrochloric acid solution \((0.1\text{mole L}^{-1})\):- Transfer 0.826 ml from concentrated Hydrochloric acid \((37 \text{%, specific gravity } 1.184)\) to 100 ml volumetric flask and diluted to the mark with distilled water.

**Recommended procedure**

Batch experiments were conducted by the addition of equal volume \((1.25\text{ml})\) of \((0.001\text{mole L}^{-1})\) potassium ferricyanide and \((0.002\text{mole L}^{-1})\) of Ferric chloride solutions to 5ml volumetric flask. Then 0.2 ml \((4\text{ drops})\) of \((0.1\text{mole/L})\) hydrochloric acid solution with 1ml of uric acid was added. The volume of the mixture was completed to the mark and heated to 50°C for 5 minutes, the absorbance of charge transfer complex formed at range 200-1100 nm was recorded. Various parameters effect experiments were conducted to evaluate the influence of six parameters selected for this study including Hydrochloric acid volume by changing its volume in the range of \((0.25 - 2\text{ml})\), potassium ferricyanide volume by varying its volume in the range of \((0.1 - 0.5\text{ml})\), Ferric chloride volume effect was studied in the range of \((0.25-2\text{ml})\). Also, the effect of temperature on the charge transfer complex formation controlled between \((30-90\text{°C})\) in water bath was studied. The reaction time between \((2-15\text{minutes})\) was evaluated to reach the maximum absorbance. Finally, the sixteen order of four additives \((\text{uric acid, potassium ferricyanide, ferric chloride, and hydrochloric acid})\) were studied by changing with a different order. Standard calibration curve for uric acid was constructed in the range of \((2-260 \mu\text{g mL}^{-1})\) concentrations.

**Determination of uric acid in live samples:**

**Blood serum samples:**- After preparation of standard solutions, the absorbance were recorded at optimum conditions controlled previously for plotting calibration curve, the absorbance of twenty serum samples for gout patients separated by centrifuge at \((3500 \text{cycle/minute})\) for 5 minutes was recorded.

**Urine samples:**- After preparation of standard solutions, the absorbance were recorded at optimum conditions controlled previously for plotting calibration curve, the absorbance of twenty urine samples for gout patients were recorded.

**Results and Discussion**

**Characterization of charge-transfer complex**

Potassium ferricyanide and ferric chloride in hydrochloric acid medium \((\text{figure 1(a)})\) forms a charge transfer complex with uric acid which is measurable spectrophotometrically at \(\lambda_{\text{max}}=752 \text{ nm (figure 1(b))}\).
As can be seen from figure 1 (a and b), the absorbance of yielded charge transfer complex shows the best wavelength is located at $\lambda_{\text{max}}=752$ nm, which shows negligible absorbance at corresponding $\lambda_{\text{max}}$ for the blank.

**Optimization conditions**

Initial experiments were conducted towards the optimization of various conditions involving the volume of potassium ferricyanide, hydrochloric acid, and ferric chloride reagents, temperature and reaction time to establish the maximum sensitivity for the charge transfer complex required for uric acid determination. The experiments were done by varying one parameters at the same time fixing the other parameters and the absorbance was measured at $\lambda_{\text{max}}=752$ nm against reagent blank.

As cited in table 1, the addition of potassium ferricyanide solution increased the absorbance up to 1.25 ml, further addition resulted in a decrease in the absorbance. Therefore, 1.25 ml was chosen for further experiments. The other parameter studied was the volume of hydrochloric acid, it was found that 0.3 ml gives the best absorbance. Thus, 0.3 m was adequate for the maximum absorbance. Ferric chloride solution volume absorbance increased and reached to a plateau between (1.5 and 1.25ml). However, 1.25 ml was selected due the minor difference.
Table 1. The study of optimum parameters

<table>
<thead>
<tr>
<th>Optimum reactant volume study</th>
<th>Optimum temperature</th>
<th>Optimum reaction time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium ferricyanide (ml)</td>
<td>Absorbance (ml)</td>
<td>Absorbance (ml)</td>
</tr>
<tr>
<td>Hydrochloric acid (ml)</td>
<td>Absorbance (ml)</td>
<td>Absorbance (ml)</td>
</tr>
<tr>
<td>Ferric chloride (ml)</td>
<td>&lt;C</td>
<td>Absorbance (ml)</td>
</tr>
</tbody>
</table>

| 0.25 | 0.526 | 0.1 | 0.547 | 0.25 | 0.359 | 30 | 0.382 | 2 | 0.759 |
| 0.5  | 0.830 | 0.2 | 0.563 | 0.5  | 0.710 | 40 | 0.391 | 4 | 0.806 |
| 0.75 | 0.884 | 0.3 | 0.584 | 0.75 | 1.021 | 50 | 0.430 | 6 | 0.872 |
| 1    | 0.891 | 0.4 | 0.504 | 1    | 1.090 | 60 | 0.477 | 8 | 1.022 |
| 1.25 | 0.940 | 0.5 | 0.439 | 1.25 | 1.403 | 70 | 0.448 | 10| 0.985 |
| 1.5  | 0.863 | 1.5 | 1.406 | 80   | 0.412 | 12 | 0.944 |
| 1.75 | 0.730 | 1.75| 1.288 | 90   | 0.370 | 14 | 0.439 |
| 2    | 0.625 | 2   | 1.090 |      |       |   |       | 15| 0.125 |

The influence of temperature and reaction time on the charge transfer complex formation was studied within the temperature range 30 to 80 °C as can be seen in table 2, the absorbance increase with increasing temperature up to 60 °C which gave the maximum absorbance. Higher temperature resulted in a decrease in the absorbance. Reaction time is a fundamental parameter for economical uric acid analysis. Consequently, a study over a period of time ranged from 2 to 10 minutes were conducted and can be seen from table 1, 8 minute was sufficient to give the maximum absorbance that was used in all subsequent study. In order to avoid the loss in color intensity, the addition of uric acid followed by the ferric chloride, potassium ferricyanide and hydrochloric acid was followed in all experiments as shown in table 2.

Table 2. The optimum order for additives.

<table>
<thead>
<tr>
<th>The order mixing additives</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>K₃[Fe(CN)₆]</td>
<td>HCl</td>
</tr>
<tr>
<td>K₃[Fe(CN)₆]</td>
<td>Uric Acid</td>
</tr>
<tr>
<td>FeCl₃</td>
<td>Uric Acid</td>
</tr>
<tr>
<td>HCl</td>
<td>K₃[Fe(CN)₆]</td>
</tr>
<tr>
<td>FeCl₃</td>
<td>HCl</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>HCl</td>
</tr>
<tr>
<td>FeCl₃</td>
<td>K₃[Fe(CN)₆]</td>
</tr>
<tr>
<td>K₃[Fe(CN)₆]</td>
<td>K₃[Fe(CN)₆]</td>
</tr>
<tr>
<td>K₃[Fe(CN)₆]</td>
<td>HCl</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>HCl</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>FeCl₃</td>
</tr>
<tr>
<td>HCl</td>
<td>HCl</td>
</tr>
<tr>
<td>K₃[Fe(CN)₆]</td>
<td>FeCl₃</td>
</tr>
<tr>
<td>HCl</td>
<td>Uric Acid</td>
</tr>
<tr>
<td>HCl</td>
<td>FeCl₃</td>
</tr>
<tr>
<td>HCl</td>
<td>K₃[Fe(CN)₆]</td>
</tr>
</tbody>
</table>
Calibration graph, accuracy and precision

Under optimum conditions, calibration graph was plotted between standard solutions over the range (2-200) μg mL⁻¹ and its absorbance at λ_max = 752 nm as shown in figure 2. The analytical data for uric acid determination illustrate in table 2, shows the limit of detection of 0.1569 and recovery average of 98.682.

![Calibration graph for uric acid determination.](image)

Table 3. Accuracy and precision for uric acid analysis.

<table>
<thead>
<tr>
<th>Uric acid Conc. (μg.mL⁻¹)</th>
<th>Relative Error R.E.%</th>
<th>Percentage Recovery Rec.%</th>
<th>Average Recovery %</th>
<th>Standard Deviation S.D.</th>
<th>Relative Standard Deviation % R.S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taken</td>
<td>Found*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>9.967</td>
<td>-0.033</td>
<td>99.676</td>
<td>0.148</td>
<td>0.755</td>
</tr>
<tr>
<td>50</td>
<td>49.225</td>
<td>0.775-</td>
<td>98.540</td>
<td>98.682</td>
<td>0.251</td>
</tr>
<tr>
<td>100</td>
<td>97.831</td>
<td>-2.169</td>
<td>97.831</td>
<td>0.362</td>
<td>4.376</td>
</tr>
</tbody>
</table>

*Average of three determinations.

Application of the adapted method for determination of uric acid in live samples: The charge transfer complex method was effectively applied for uric acid determination in live sample including twenty men biological samples (serum and urine). The results indicated in table 4.
Table 4. The analysis of uric acid in live samples.

<table>
<thead>
<tr>
<th>Concentration (µg mL⁻¹)</th>
<th>Sample number</th>
<th>Concentration (µg mL⁻¹)</th>
<th>Sample number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>Serum</td>
<td>Urine</td>
<td>Serum</td>
</tr>
</tbody>
</table>

Conclusion

The literatures listed a variety analytical techniques for determination of uric acid, this method were simple, accurate, rapid, precise, sensitive, and don’t needs additional steps or special working conditions. Moreover, charge transfer complex formed owing good stability in the solution.

Acknowledgment: The authors would like to thank Mustansiriyah University-Baghdad-Iraq for its support in the present work.

Ethical approval: This study was permitted by the gout patients and all the experiments were done in compliance with the statements on informed consent by all patients.

Conflicts of Interest: There are no conflicts to declare.

Source of Funding: This work was self-funding.

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6. Hairul Hisham Hamzah1 ZMZ, , Nor Lailatul Wahidah Musa,, Yun-Chun Lin, and Emma Trimbee. Spectrophotometric Determination of Uric Acid in Urine Based-Enzymatic Method Uricase with 4-Aminodiphenylamine Diazonium


Control of Risk in the Process Loading / Unloading In. Pelindo Iii Surabaya

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¹Department of Occupational Health and Safety, Faculty of Public Health, Airlangga, University, Indonesia

Abstract

PT. Pelindo III Surabaya is a company engaged in service providers. One of them is loading/unloading services. The results of preliminary observations made at PT. Pelindo III Surabaya shows that container loading/unloading workers do not follow standard operating procedures in the workplace such as not using safety helmets, safety vest, and safety shoes. Neat and dirty workplace conditions can increase potential hazards such as falls and tripping material and damage to the goods. This can threaten the safety and disturb the health of workers loading/unloading containers at PT. Pelindo III Surabaya. This study aims to analyze the risk assessment on loading/unloading work at PT. Pelindo III Surabaya. This research is an observational study with design crossectional. The study population was the workers in the loading/unloading section at PT. PELINDO III Surabaya as many as 20 people. Data that has been obtained from observations and interviews are processed and analyzed descriptively, namely by describing in real terms the object of research and the conditions at the research site. The results of the study show the process of loading/loading PT. PELINDO III Surabaya has 5 types of hazard risks in high-risk categories, 12 types of hazard risk, medium risk categories and 3 types of hazards in low-risk categories. The company is expected to provide and require workers to use body harnesses as additional PPE for workers who are above the container during the process cargo during.

Keywords: risk assessment, loading/unloading

Introduction

Companies that provide loading/unloading services are one of the most important companies in the transportation of goods and services. Unloading/loading services are useful for transporting goods in various places including ports. Transportation services increasingly offer or provide facilities such as container terminals, loading/unloading equipment, and containers themselves.

Data on occupational accidents (including death) that occurred in loading and unloading workers (TKBM) from 2004 to 2007 were 110 people. Details of the incidence of workplace accidents loading and unloading workers (TKBM) are: in 2004 as many as 29 people (4.11%), in 2005 as many as 44 people (5.6%), in 2006 as many as 17 people (2.21%) and in 2007 as many as 20 people (2.65%)¹.

Things that affect the risk include the type of work, job location, potential harm due to hazards in the workplace, potential harm due to contractor activities, simultaneous work by several contractors, duration of work, experience and expertise of contractors².

One of the loading/unloading service providers, including PT. Pelindo III Surabaya which plays a role in carrying out loading/unloading and is one of the largest container companies in Indonesia. Activities loading/unloading occurs increases significant every year where there was an increase of 0.64% in 2013.

The number of workers loading/unloading port of
Tanjung Perak in 2004 as many as 705 people, in 2005 as many as 756 people, in 2006 as many as 796 people and in 2007 as many as 756 people.

One effort that can be done to prevent work accidents is by implementing a Risk Assessment which is a process to evaluate the risks arising from a hazard by considering the adequacy of the controls owned and determining whether the risks are acceptable or not.

The danger of risk assessment needs to be done so that the amount of risk that can occur is known, so that after the hazard is identified a risk assessment is carried out to find out how big the risk is (how big the risk).

Risk assessment is the process of risk analysis and evaluation to determine the level of risk and level of risk and determine whether the risk is acceptable or not. Risk Assessment is an attempt to calculate the amount of risk and determine whether the risk is acceptable or not.

All risks that have been identified and assessed must be controlled, especially if the risks are considered to have a significant impact or cannot be properly controlled in terms of various aspects such as financial, practical, human and other operations.

Based on this, research was conducted with the aim of analyzing risk assessment which included activities to identify hazards at loading/unloading work at PT. Pelindo III Surabaya, analyze risk assessment and analyze risk control at loading/unloading work at PT. Pelindo III Surabaya.

**Methods**

This study was an observational study that was descriptive in nature with design cross-sectional to analyze the risk assessment on loading/unloading work at PT. Pelindo III Surabaya. This research was conducted in December 2014.

The population of this study was unloading/loading workers PT. PELINDO III Surabaya with a total of 20 workers. The sample size taken in the study used a total population of 17 workers, 2 officers of Occupational Safety and Health (K3), and 1 operational supervisor.

The variables examined in this study were hazard identification, risk assessment, and risk control. There are no dependent variables and independent variables because this research is a descriptive study.

Primary data is collected by conducting field observations and interviewing respondents. Observation is carried out by making observations directly by seeing, and recording all the conditions in the workplace both regarding process activities in the loading/unloading section, materials, tools, number of workers, working environment conditions, work methods, control technology, personal protective equipment.

The instruments used for data collection in this study include Form Job Safety Analysis (JSA); Risk assessment matrix sheet; sheet Risk Assessment Table to carry out a risk assessment; Interview guide; Observation sheet.

**Data analysis carried out include:**

Identifying the existing material at the loading/unloading sub-unit of PT. PELINDO III Surabaya using the technique Job Safety Analysis (JSA) that is entered into the risk assessment matrix and observes the hazard for calculation using a risk assessment matrix table.

After identification, the risk assessment calculation is complete, where the hazards that have been classified are assessed based on exposure values, opportunities, and consequences. At the risk assessment stage, there is a perception of equality between researchers and PT. PELINDO III Surabaya in determining exposure events, opportunities, and consequences based on the theory put forward by Suardi (2007), in order to get the results correctly and precisely which has been operationalized by the company. The calculations from each aspect can be seen as follows:

**Table 1. Determining the Opportunity (L) in the Risk Assessment at PT. PELINDO III Surabaya**

<table>
<thead>
<tr>
<th>Choice of Opportunities</th>
<th>Operational Definition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very often</td>
<td>Can occur at any</td>
<td>1</td>
</tr>
<tr>
<td>Often</td>
<td>Can occur periodically</td>
<td>0.6</td>
</tr>
</tbody>
</table>
Cont. Table 1. Determining the Opportunity (L) in the Risk Assessment at PT. PELINDO III Surabaya

<table>
<thead>
<tr>
<th>Kinds of Opportunity</th>
<th>Operational Definition of</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderate</td>
<td>can occur in certain conditions</td>
<td>When there is work every day</td>
</tr>
<tr>
<td>Rarely</td>
<td>Can occur under certain conditions</td>
<td>At when there is work within a few days of the week</td>
</tr>
<tr>
<td>Very Rarely</td>
<td>Allows never to occur</td>
<td>very rarely the existence of work (can be counted in a few weeks)</td>
</tr>
</tbody>
</table>

Source: Suardi (2007)

Table 2. Determining the consequences (K) of the Risk Assessment at PT. PELINDO III Surabaya

<table>
<thead>
<tr>
<th>Kinds of Consequences of</th>
<th>Operational Definition of</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatal</td>
<td>Can directly lead to workplace accidents and result in death</td>
<td>20</td>
</tr>
<tr>
<td>Major</td>
<td>Can cause direct workplace accidents but not death (Severe injury)</td>
<td>10</td>
</tr>
<tr>
<td>Medium</td>
<td>Can cause workplace accidents but can still be handled by company clinics</td>
<td>5</td>
</tr>
<tr>
<td>Minor</td>
<td>Can cause work accidents but is still said to be a minor injury (can be handled using a first aid kit)</td>
<td>2</td>
</tr>
<tr>
<td>Not Significant</td>
<td>Still said to be safe</td>
<td>1</td>
</tr>
</tbody>
</table>

Source: Suardi (2007)

Table 3. Determining Exposure (E) to Risk Assessment at PT. PELINDO III Surabaya

<table>
<thead>
<tr>
<th>Types of Exposure to</th>
<th>Operational Definitions</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous</td>
<td>for 12 hours or more a day</td>
<td>10</td>
</tr>
<tr>
<td>Periods More or</td>
<td>less for 4-8 hours a day</td>
<td>6</td>
</tr>
<tr>
<td>Specific For</td>
<td>more or less 2-3 hours a day</td>
<td>3</td>
</tr>
<tr>
<td>Not regular for</td>
<td>more than 1 hour a day</td>
<td>2</td>
</tr>
<tr>
<td>Rarely</td>
<td>As long as there are only jobs</td>
<td>1</td>
</tr>
</tbody>
</table>

Source: Suardi (2007)

The results of these calculations are combined through a risk level matrix with semi-quantitative risk analysis techniques which will then be obtained the level of risk ranging from low risk to high risk, then evaluated to determine whether the risk is acceptable, accepted by conditions, or not accepted. This study uses a 3D model risk assessment so that the risk value category loading/unloading activities can be seen in Table 4.
Table 4. Definition of 3D Risk Assessment Model

<table>
<thead>
<tr>
<th>Definition of</th>
<th>Exposure</th>
<th>Opportunities</th>
<th>Consequences</th>
<th>Risk Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Continuous</td>
<td>Very often</td>
<td>Fatal</td>
<td>20 E &gt; 20</td>
</tr>
<tr>
<td></td>
<td>Periodic</td>
<td>Often</td>
<td>Major</td>
<td>10 H 11-20</td>
</tr>
<tr>
<td></td>
<td>Specific</td>
<td>Moderate</td>
<td>Medium</td>
<td>5 M 3-10</td>
</tr>
<tr>
<td></td>
<td>Irregular</td>
<td>Rarely</td>
<td>Minor</td>
<td>2 L &lt;3</td>
</tr>
<tr>
<td></td>
<td>Rarely</td>
<td>Very rare</td>
<td>Not Significant</td>
<td>1</td>
</tr>
</tbody>
</table>

* Source: Book of occupational health and safety management systems, Rudi Suardi (2007)

After carrying out the analysis and risk level the next stage is to do risk control and classifying risk control whether it feels sufficient or not fulfilling. Risk control is said to be sufficient if it meets ≥ 2-4 controls, both substitution, technical, administrative and PPE control. Risk control is said to be lacking if it only meets the criteria of one type of control that exists.

Results

General Description of Unloading / Loading at PT. PELINDO III Surabaya

Port of Tanjung Perak is one of the port gates of eastern Indonesia, becoming the center of collectors and distributors of goods to Eastern Indonesia. Its strategic location makes Tanjung Perak Port the center of interinsular shipping in Eastern Indonesia and becomes the second largest port after the Tanjung Priok Port in Jakarta.

Geographically, the location of Tanjung Perak Port in Surabaya is at position 112°44’100” -112°32’40” East Longitude and 7°11’40” -7°13’20” South Latitude precisely located at the North of Surabaya City and with a wide overall, 2,218 ha. Tanjung Perak Port Surabaya has 8 terminals, including Emerald Terminal, Berlin Terminal, Patchouli Terminal, Mirah Terminal, Passenger Terminal, Surabaya Container Terminal, Roro Terminal, Kalimas Terminal.

In total, Surabaya Container Terminal has unloaded/loaded 192,047 Twenty feet Equivalent Units (TEU’s) in early 2014, while in the same period in 2013 there were 153,249 Twenty feet Equivalent Units (TEU’s). For the past four months, domestic loading/unloading services have reached 9,000 Twenty feet Equivalent Units (TEU’s), so that on average 2,000obtained on average Twenty feet Equivalent Units (TEU’s) are.

Hazard Identification in the Container Loading / Unloading Process at PT. PELINDO III

The process of identifying hazards on loading/unloading work container PT. PELINDO III Surabaya is carried out by taking into account the work steps, hazards arising from activities in the loading/unloading process, and the impact resulting from the danger that arises.

Hazard identification results on container loading/unloading work at PT. PELINDO III Surabaya that has been done is shown in Table 5.
### Table 5. Hazard Identification in the Unloading / Loading Section of PT. Pelindo III Surabaya 2015

<table>
<thead>
<tr>
<th>Process</th>
<th>Work</th>
<th>Hazards</th>
<th>Impacts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cargodoring</td>
<td>lifts on</td>
<td>Terpleset</td>
<td>wounds, fractures, died</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fell into the sea</td>
<td>Drowned</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tripping</td>
<td>injuries, fractures, died</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Affected container</td>
<td>Death</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Caught container</td>
<td>wounds, broken bones, death</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sling broke</td>
<td>wounds, broken bones, died</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sandwiched hook</td>
<td>wounds, fractures</td>
</tr>
<tr>
<td>Shift ground</td>
<td>Crushed container</td>
<td>died world</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sandwiched container</td>
<td>fractures, died</td>
<td></td>
</tr>
<tr>
<td>Trucking</td>
<td>Container fall</td>
<td>died world</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Collision</td>
<td>Injuries, fractures, died</td>
<td></td>
</tr>
<tr>
<td>Stevedoring</td>
<td>lifts off</td>
<td>Terpleset</td>
<td>wounds, fractures, died</td>
</tr>
<tr>
<td></td>
<td>Tripped</td>
<td>Wounds, fractures</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stricken by container</td>
<td>Passed by</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pinned container</td>
<td>A broken bone, died</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stacking</td>
<td>Container fell</td>
<td>A broken bone, died</td>
</tr>
<tr>
<td></td>
<td>Stuck by container</td>
<td>Death of</td>
<td></td>
</tr>
</tbody>
</table>

Activities to identify hazards include diagnosing and finding hazards in parts of the system and subsystems, the sequence of activities and also calculating the possibility of arising and the consequences that these hazards will produce. Doing hazard identification well will result in good risk management.

**Hazard Risk Analysis at Container Loading / Unloading Work at PT. PELINDO III Surabaya**

The results of the study regarding the risk assessment of hazards at loading/unloading work at PT. PELINDO III Surabaya is shown in Table 6. The results of exposure multiplication, opportunities and consequences are then evaluated using a risk matrix so that it can be concluded that the danger is included in the category of danger with low, medium or high risk.

Risk assessment is the process of risk analysis and evaluation to determine the level of risk and level of risk and determine whether the risk is acceptable or not. (Ramli, 2010). Risk assessment in the loading/unloading section at PT. PELINDO III Surabaya is based on data in the field conducted by observing and distributing questionnaires to all permanent workers.

**Stevedoring**

1.5 risk value is owned by a dripped hazard and stumbles on activities elevator off. The danger of falling container, sandwiched container, container falling and falling container on lift off and stacking value risk 6.

**Receiving / Delivery The**

the danger of falling container and container has a value of risk 6. The danger of collisions between containers is in the low category with a risk value of 1.5.

Activities receiving/delivery of services that activity in the form of work takes a pile of goods / Line I place the accumulation of goods or yard goods and deliver the goods to be composed on the vehicle/conveyance tightly in the dooryard goods ashore or otherwise.
In the process receiving/delivery there is only one job, namely, lift on with a low-risk category with a value of 1.5 and a moderate risk category with a value of 6.

Low risk in the process receiving is due to the low exposure value and the possibility of an accident high. Risk is generally acceptable but still needs to be reviewed.

**Table 6. Hazard Risk Assessment in the Loading / Unloading Process at PT. PELINDO III Surabaya Year 2015**

<table>
<thead>
<tr>
<th>Process</th>
<th>Work</th>
<th>Danger</th>
<th>Impact of</th>
<th>Risk Assessment Risk</th>
<th>Value Risk</th>
<th>Level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>E x L x K</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cargodoreing</td>
<td>Lift on</td>
<td>Plastered Wounds, fractures, dies</td>
<td>6 0.1 5 3</td>
<td>Risk of Being</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Falling into the sea Sinking</td>
<td>6 0.05 10 3</td>
<td>Medium Risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tripped ulcers, fractures, died</td>
<td>6 0.1 5 3</td>
<td>Medium Risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Affected container death</td>
<td>6 0.1 20 12</td>
<td>High Risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wedged container ulcers, fractures, mortality</td>
<td>6 0.3 10 18</td>
<td>High Risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sling broke Luka, fracture, death</td>
<td>6 0.05 10 3</td>
<td>Risk of Being</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>sandwiched hook wound</td>
<td>6 0.3 5 9</td>
<td>Risk Medium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shift ground</td>
<td></td>
<td>overwitten container Died</td>
<td>6 0.1 20 12</td>
<td>High Risk of</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>sandwiched container Fracture died</td>
<td>6 0.1 20 12</td>
<td>High Risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trucking</td>
<td>Container fall died</td>
<td>6 0.1 20 12</td>
<td>High Risk of</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Collision Injuries, fractures, died</td>
<td>6 0.1 5 3</td>
<td>Medium Risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stevedoring lifts off</td>
<td>Terpleset Wounds, fractures, died</td>
<td>3 0.1 5 1.5</td>
<td>Low Risk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tripped Luka, fracture</td>
<td>3 0.1 5 1.5</td>
<td>Low Risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Affected container Died</td>
<td>3 0.1 20 6</td>
<td>Risk of Being</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sandwiched Container Broken bones, died</td>
<td>3 0.1 20 6</td>
<td>Risk Moderate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stacking</td>
<td>Container fell Broken bones, died</td>
<td>3 0.1 20 6</td>
<td>Risk of Being</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Affected by Container Died</td>
<td>3 0.1 20 6</td>
<td>Risk Medium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Receiving / Delivery</td>
<td>lifts on Crushed container Died</td>
<td>3 0.1 20 6</td>
<td>Risk Medium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Container fell Broken bones, died</td>
<td>3 0.1 20 6</td>
<td>Risk Medium</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Collision between containers Broken bones, died</td>
<td>3 0.1 5 1.5</td>
<td>Low Risk</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Hazard of Risk Control in the Unloading / Loading Process at PT. PELINDO III SURABAYA

Potential hazards that will be received by loading and unloading workers (TKBM) have a high-risk level are crushed and pinched containers inactivities elevator on, crushed containers inactivities shift ground and pinned containers and containers fall activities trucking.

Control with PPE that has been carried out by PT. PELINDO III includes procurement of gloves, helmets and safety shoes for workers, especially those who carry out loading/unloading activities above the container or crane.

Risk control implemented by PT. PELINDO III Surabaya is categorized enough because it has made a combination of two hierarchies, namely administrative control, and the use of personal protective equipment (PPE)⁶.

The risks that are known to be large and the potential consequences must be managed appropriately, effectively and in accordance with the capabilities and conditions of the company³.

Risk control using the approach Long Term Gain in Tarwaka’s book (2008) regarding the hierarchical approach to control, namely long-term and permanent oriented control starting from engineering control, isolation or restriction, administration and the most recent is the use of personal protective equipment (PPE)⁷.

Control by procuring PPE has been applied at PT. PELINDO III Surabaya to minimize work accident rates.

Conclusion

The conclusions that can be taken based on the results of this study are:

Potential hazards identified in the loading/unloading section at PT. PELINDO III Surabaya there are 20 types of potential hazards from 6 jobs in 3 loadings/unloading processes.

Potential hazards identified in loading/loading PT. PELINDO III Surabaya found 5 dangers of high-risk categories, 12 hazards of moderate risk categories and 3 hazards of low-risk categories.

Conflicts of Interest: All authors have no conflicts of interest to declare.

Source of Funding: The source of this research costs from self.

Ethical Clearance: The study was approved by the Institutional Ethical Board of Faculty of Public Health, Hasanuddin University.

All subjects were fully informed about the procedures and objectives of this study each subject prior to the study signed an informed consent form.

References

An Investigation into the Effect of Multimedia Training on the Knowledge and Self-Efficacy of Children with Asthma

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Abstract

Introduction: Asthma is one of the most common chronic diseases among children is a global health issue and its rising trend has caused concern about the health system. Therefore, this study was conducted to investigate the effect of multimedia education on the knowledge and self-efficacy of children with asthma in Bushehr.

Method: semi-experimental study. The research community included all children under the age of 12 years with asthma. The samples were selected by available method of 50 people and then divided into two experimental and control groups by simple random method. The data collection tools included questionnaires of demographic data, knowledge and self-efficacy in children with asthma. Validity and reliability of the instruments were measured and then multimedia training was run for the experimental group. Data analysis was performed by SPSS 18 and Mann–Whitney U test.

Findings: the mean scores of knowledge and self-efficacy of the research samples in the experimental group increased after multimedia training, so there was a statistically significant difference between the two groups (p <0.001).

Conclusion: Education of children with asthma through multimedia has led to increasing knowledge and self-efficacy. Therefore, considering the effectiveness of multimedia education in children training with asthma, it is recommended that this educational method are used for other chronic childhood diseases.

Keywords: self-efficacy, knowledge, multimedia, asthma, children

Introduction

Chronic pulmonary diseases are among the commonest diseases causing the death and disablement of human societies which asthma is one of the commonest [1]. According to the statistics, 3-35% of people in the world (300 million individuals) suffer from asthma [2-3] that 100 million more patients may be added to this statistic until 2025 [4-5]. Asthma is the commonest chronic disease during childhood, the first reason of receiving in emergency operation centers and the third reason of hospitalizing children under 15 [6-8]. Furthermore, this disease is the important factor of absence from school among chronic diseases [9]. Children with asthma are more absent from school and more awake during the night [10-15]. The continuation of weak results in treating children with asthma has led to various educational interruptions in order to improve the care, therefore, the need to provide innovative methods to improve asthma care is increasingly felt [15]. Educational media is one of these innovative methods. The most important reasons of using educational media are as follows: “developing effective communication”, “providing motivation for

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learning”, “forming faster, deeper, and more stable learning”, “economizing teaching and learning time”, “adapting to multiple intelligences”, and “forming impossible learning experiences”. The term multimedia was proposed since 1950 and it was attempted to combine several mediums to enhance the quality of training [16]. Multimedia training as a new educational method is easily, widely, and attractively performed by transferring educational concepts and contents along with text, voice, image, and video and today, it is widely used in order to transfer concepts during childhood and teenage years [16]. This new educational programme can facilitate the process of decision-making by empowering patients to have an active role in selecting health-based programmes [17].

**Method**

The present study is quasi-experimental and it was conducted on 50 children with asthma in Bushehr. The population included all children with asthma under 12 and sampling was first of convenience type, then samples were divided into two groups of intervention and control using random allocation. The entry criteria to this study include all children with asthma (girl and boy) under 12 with the final diagnosis of a doctor, the tendency of children and their parents to participate in the study, having no history of chronic heart, vascular, liver, and kidney diseases, and being able to participate in the empowerment plan. The exit criteria to this study include the tendency of children and their parents to exit the study, the membership of parents in the health team, and the participation of study samples in similar researches. The sample volume for alpha 0.05 and power of the test (80%), for standard deviation of 10, and detecting the difference of 8 scores between two groups was equal to 25 in each group. The tools of this research include the 15-question questionnaire of demographic information about the child and their parents completed by the child as well as their parents if needed, the 8-question questionnaire of measuring the knowledge of child with asthma, and the 10-question questionnaire of measuring the child’s knowledge about allergens including 3 video questions and the checklist of assessing child’s self-efficacy about using the asthma spray. The validity and reliability of these questionnaires were evaluated in a research aimed at investigating the effect of family-centered empowerment model on the quality of life in school-age children with asthma [4].

This study was conducted through three stages:

A. The stage before Intervention:

When the parents participated, the researcher introduced himself and explained the aims, importance, and method of the study and by giving them written consent based on how to intervene and complete the questionnaires the researcher assured them that the data and information are confidential and obtained parents’ written consent and children’s verbal consent.

b. The Intervention Stage

The research-made CD contained teaching the two essential steps of knowledge increase and self-efficacy increase as well as some information about pathophysiology of disease, physiology, symptoms, environmental and indoor allergens, proper use of spray, nutrition, medicine, etc. Then the CD was granted to some allergy and asthma specialists in order to determine its validity and their viewpoints were evaluated. After the confirmation of allergy and asthma specialists and scientific editing by one of the faculty members of Bushehr school of Nursing and Midwifery, the CD was applied to the samples through two steps.

c. The Stage After Intervention

For measurement, the data was analyzed using spss 18. In this study, descriptive statistics included frequency, frequency percentage, mean, and standard deviation. Moreover, in order to compare the knowledge and self-efficacy scores of studied samples in both intervention and control groups, Non-Parametric Mann-Whitney Test was applied.

**Findings**

The mean (and standard deviation) of children’s age in the test group was 8.80 (and 1.936) years old and in the control group was 8.86 (and 1.406) years old and the mean (and standard deviation) of history of disease in the test group was 3.36 (and 0.810) years and in the control group was 3.36 (and 1.15) years. 72% of children were at the middle stage of disease severity and 28% were at the mild stage. Most children’s families showed the history of asthma (52%), 54% reported that they smoked indoors, and 48% (the highest percentage) had the history of asthma for three years and more. Most children’s fathers in the test group (48%) and control group (36%) were self-employed. Moreover, most children’s mothers in the test group (88%) and control group (96%) were
housewives. Table 1 indicates the mean and standard deviation for the knowledge of children with asthma in the test group and control group before and after the intervention. In this regard, the results of Mann-Whitney Test between test group and control group before the intervention showed no significant statistical difference, however, after the intervention, a significant difference was found (p<0.001). Table 2 indicates the mean and standard deviation for the self-efficacy of children with asthma regarding the use of inhaled spray in the test group and control group before and after the intervention. In this regard, the results of Mann-Whitney Test between test group and control group before the intervention showed no significant statistical difference, however, after the intervention, a significant difference was found (p<0.001). Table 3 indicates the mean and standard deviation for the knowledge of children with asthma regarding the recognition of allergens in the test group and control group before and after the intervention. In this regard, the results of Mann-Whitney Test between test group and control group before the intervention showed no significant statistical difference, however, after the intervention, a significant difference was found (p<0.001).

**Table 1. Comparing the scores for the knowledge of children with asthma before and after the intervention using multimedia training method between the test group and control group**

<table>
<thead>
<tr>
<th>Component</th>
<th>Test</th>
<th>Control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before the intervention</td>
<td>After the intervention</td>
<td>Before the intervention</td>
</tr>
<tr>
<td>Knowledge</td>
<td>Mean (standard deviation)</td>
<td>Mean (standard deviation)</td>
<td>Mean (standard deviation)</td>
</tr>
<tr>
<td></td>
<td>4.28 (1.94)</td>
<td>7.28 (0.93)</td>
<td>4.04 (1.09)</td>
</tr>
</tbody>
</table>

**Table 2. Comparing the scores for the self-efficacy of children with asthma regarding the use of inhaled spray before and after the intervention using multimedia training method between the test group and control group**

<table>
<thead>
<tr>
<th>Component</th>
<th>Test</th>
<th>Control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before the intervention</td>
<td>After the intervention</td>
<td>Before the intervention</td>
</tr>
<tr>
<td>Self-efficacy</td>
<td>Mean (standard deviation)</td>
<td>Mean (standard deviation)</td>
<td>Mean (standard deviation)</td>
</tr>
<tr>
<td></td>
<td>3.92 (1.70)</td>
<td>8.44 (1.04)</td>
<td>4.33 (1.73)</td>
</tr>
</tbody>
</table>
Table 3. Comparing the scores for the knowledge of children with asthma regarding the recognition of allergen before and after the intervention using multimedia training method between the test group and control group

<table>
<thead>
<tr>
<th>Component (recognition of allergen)</th>
<th>Test Before the intervention</th>
<th>Test After the intervention</th>
<th>Control Before the intervention</th>
<th>Control After the intervention</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (standard deviation)</td>
<td>9.75 (3.73)</td>
<td>16.60 (1.82)</td>
<td>10.84 (3.53)</td>
<td>12.16 (3.70)</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

Discussion

Asthma is one of the commonest chronic diseases in children. Although mortality from asthma has decreased at present, this disease is one of the commonest reasons for hospitalizing children in advanced countries and the recurrence symptoms of asthma have lowered children and their families’ quality of life to a large extent [18]. The results of researches indicate that training programmes of asthma should be considered as a part of routine care in order to improve treatment care, reduce treatment costs, and lessen patients’ unwellness [19]. The results of the present study indicated that there is a significant difference between the mean of child’s knowledge before and after the intervention (p<0.001), therefore, multimedia training increased child’s knowledge in the intervention group after training. In the present research, comparing the scores of quality of general life before and after the intervention in the physical dimension indicated a significant difference in the direction of improving child’s quality of general life (physical dimension) in the intervention group (p<0.001). The knowledge about asthma refers to the ability to differentiate irritants, understand the role of lifesaving treatments, and develop programmes to manage this disease when intensifies [20]. The results of researches around the world indicate that patients with asthma have little knowledge about their disease. In the study conducted by Gibson (as cited in Sharifi), it was concluded that knowledge about asthma is little among students and teachers [21]. On the other hand, the findings of researches indicate that using new educational methods for training children with asthma can increase their knowledge [8]. For example, in a research conducted by Keulers et al., the results showed that the scores of patients’ knowledge were increased significantly after training using a computer-based programme (p= 0.001). Their results also indicated that training through computer is an excellent method to increase the knowledge about the disease and treatment [22]. In another study conducted by Krishna et al. on children with asthma, they found that using multimedia technology in training children with asthma improves the consequences of health in the intervention group [9]. By comparing the mean difference of children’s self-efficacy scores regarding the use of inhaled spray between test group and control group before and after the intervention, the results showed that there is a significant statistical difference in the direction of enhancing the self-efficacy of children with asthma in the test group (p<0.001). The low levels of self-efficacy lead to more hospitalization [20], however, using modern methods such as multimedia in training can enhance self-efficacy and performance [23]. According to Bandura, self-efficacy is related to previous successes in similar situations or experiencing other people’s successes [24] and based on the theory of social learning, the knowledge and skill obtained in simulated environments are transferable to real situations and multimedia has the special capacity and potential to transfer the information for these patients [25]. Self-efficacy in relation to asthma refers to the self-confidence to interpret the disease and its symptoms exactly as well as following appropriate care programmes [26] and in our study, self-efficacy
refers to children’s empowerment to identify different environmental and indoor allergens, choosing good food, exercise, and medicine, in fact, the most important aspect emphasized in this study is how to use the inhaled spray accurately. Using the tool and spraying it accurately to release aerosol into airways is crucial. The results of studies indicate that usually the accurate technique of inhaling even after training children is often lower than the desired level [18]. The results of our study showed that multimedia training could motivate children with asthma to use the inhaled spray accurately. Furthermore, in a research conducted by Chenge et al. on children with asthma, it was found that children who participate in asthma training programmes can improve their physical and social activities that lead to the enhancement of self-efficacy and self-management. In addition, according to their findings, training can reduce the symptoms of this disease and improve the quality of life [12]. In the research conducted by Robin et al., it was indicated that a computer-based educational programme enhances patients’ performance and behavior, which is effective in reducing the number of visiting treatment centers [25]. Generally, asthma has a deep effect on patients’ life and performance and influences their group activities and social performance [11], however, children who participate in asthma training programmes can improve their social activities that lead to the enhancement of self-efficacy [12].

Conclusion

Regarding the modern, effective, and simple multimedia training in learning as well as its attraction for children and parents, performing this model in order to improve the results of other chronic diseases in childhood by means of multimedia training is recommended.

Acknowledgement: I deem it necessary to acknowledge the cooperation of all the personnel and doctors.

Ethical Clearance- Taken from Bushehr University of medical science committee

Source of Funding- Self

Conflict of Interest – Nil

References

based on compliance and empowerment on the quality of life of patients with asthma. Journal Of Nursing Education. 1392;2(3):1-7.


Determination of Safe Level of Benzene Concentration in Mechanics Workshop “X” Tembalang Semarang

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Abstract

Vehicles are used in community in a large number and being repaired in motorbike repair shops. The repaired motorbike at the workshop has the potential to provide benzene exposure to the mechanic, so the mechanic was also at risk of experiencing health problems due to benzene exposure. This study aimed to determine the safe concentration of benzene in Workshop X Tembalang, Semarang. This study was a descriptive research. The population in this study were mechanics at Locations 1 and 2, totally 18 people. The results of this study were quantitatively analyzed to determine the safe concentration of benzene for workers obtained from the benzene concentrations in the workplace, worker height, worker weight, rats weight, worker respiration rate, length of work, worker body surface area, mice body surface area, highest dose of toxin without effect on experimental animals (NOAEL), Km factor in animals (Animal Km), Km factors in workers (Human Km), and safe limit dose for workers (RIC). The measurement of benzene concentration at Location 1 was 0.28 ppm and Location 2 was 0.19 ppm, which means that the benzene concentration was still below the Threshold Value according to Minister of Manpower Regulation Number 13 in 2011 amounting to 0.5 ppm. This research showed that the safe limit value was 0.023 ppm. Based on the minimum risk level, the concentration of benzene everyday that can cause acute effects was 0.009 ppm and that can cause chronic effects was 0.003 ppm. These standards indicate that the concentration of benzene in the workshop has the potential to have a negative impact on the health of workers. Recommendations to workshop owners and mechanics are to periodically monitor benzene levels in the air, use personal protective equipment by all mechanics and if needed the workshop owner can conduct a health check-up for all mechanics.

Keywords : Benzene, safe level, mechanics workshop

Introduction

Motorcycles were widely used in community and increased every year in Indonesia. Data from AISI (Asosiasi Industri Sepeda Motor Indonesia) in 2015 stated that motorcycle sales in 2014 reached 7,926,104 million units1. The large number of motorbike uses can provide opportunities for motorcycle workshops to provide automotive needs services that include repair and maintenance processes that carried out by workshop mechanical workers2. Workshop mechanics often referred to as mechanics or engineers who works to repair, install, or modify the vehicles which in the work process was very high risk of exposure to hazardous chemicals3.

Benzene was a chemical that was dangerous and carcinogenic for workers which found in fuel oil4,5. The limit levels of benzene exposure according to several world organizations are 1 ppm (National Institute for Occupational Safety and Health6 and Occupational Health and Safety Assessment Series7) and 0.5 ppm (the American Conference of Governmental Industrial Hygienists8).

In Indonesia, the limit of exposure benzene in the work environment was stipulated in the threshold value (NAB) of benzene at 0.5 ppm according to the Regulation and maintenance processes that carried out by workshop mechanical workers2. Workshop mechanics often referred to as mechanics or engineers who works to repair, install, or modify the vehicles which in the work process was very high risk of exposure to hazardous chemicals3.

Benzene was a chemical that was dangerous and carcinogenic for workers which found in fuel oil4,5. The limit levels of benzene exposure according to several world organizations are 1 ppm (National Institute for Occupational Safety and Health6 and Occupational Health and Safety Assessment Series7) and 0.5 ppm (the American Conference of Governmental Industrial Hygienists8).

In Indonesia, the limit of exposure benzene in the work environment was stipulated in the threshold value (NAB) of benzene at 0.5 ppm according to the Regulation.
of the Minister of Manpower and Transmigration PER/13/MEN/X/2011 about Factor Threshold Values Physics and Chemistry at Work9.

Some research show that the concentration of benzene in the air below the normal limit, which still have negative effects such as headaches and tremors10. Determination of safe limits of benzene concentration in the work environment involves knowledge of worker breathing rate (BR), reference concentration (RfC), work-hours/day (tE), weight (Wb) and the average cancer year (70 years) and non-cancer (30 years)11.

This study aims to determine the level of safe concentration of benzene in Bengkel X in Tembalang Semarang so that it can provide recommendations to workshop owners so they can determine safe actions and protect workers from benzene exposure.

**Material and Method**

This study was a descriptive study that aims to determine the magnitude of the environmental health risk of benzene exposure to workers in Workshop X Tembalang Semarang using an environmental health risk analysis approach. The population in this study was a mechanic totaling 18 people.

The design of the research was to collect initial data, the work process, and the number of workers. Furthermore, collecting primary data was the concentration of benzene in the workplace air, length of work time, and worker weight. Secondary data collection was also carried out on experimental animals, the weight of white mice. The type of data in this study was primary data, obtained through questionnaires and observations as well as measurements of benzene in the air of the workers’ workplace.

The variables in this study were benzene concentration in the workplace, worker height, worker weight, rats weight, worker respiration rate, length of work, worker body surface area, mice body surface area, highest dose of toxin without effect on experimental animals (NOAEL), Km factor in animals (Animal Km), Km factors in workers (Human Km), and safe limit dose (RfC), and benzene concentration in safe air for workers (C safe).

Data analysis in this study was carried out by using quantitative data analysis manually to determine the safe concentration of benzene for workers in the work environment in Workshop X Tembalang Semarang.

**Findings**

1. **Characteristics and Surface Area of Animals Experiments**

Toxicity can be interpreted as the ability of poisons to cause damage if it enters the body and the location of organs susceptible to it. Toxicity was very diverse for various organisms, depending on various factors such as test species, the way poisons enter the body, the frequency and length of exposure, concentration of exposure agents, and the vulnerability of various species to pollutants. In this study, the implementation of a toxicity experimental test in white mice. This study was done considering the human response to toxicity qualitatively was the same as the response of animals, so this fact was the base of extrapolation from animal to human data.

The following are characteristic data of experimental animals.

**Table 1. Characteristics of Animal Experiments**

<table>
<thead>
<tr>
<th>Animal Experiments</th>
<th>W (kg)</th>
<th>BSA (m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1405</td>
<td>0.024165</td>
</tr>
<tr>
<td>2</td>
<td>0.1405</td>
<td>0.024165</td>
</tr>
<tr>
<td>3</td>
<td>0.1410</td>
<td>0.024223</td>
</tr>
<tr>
<td>4</td>
<td>0.1410</td>
<td>0.024223</td>
</tr>
<tr>
<td>5</td>
<td>0.1395</td>
<td>0.024050</td>
</tr>
<tr>
<td>6</td>
<td>0.1415</td>
<td>0.024165</td>
</tr>
</tbody>
</table>

Based on these data, it can be calculated the body surface area of white mice with :

Animal BSA = 0.09 x \( W^{0.67} \)

Description:

BSA : Body Surface Area (m²)

W : Weight (kg)

2. **Characteristics, Body Surface Area, and Respiratory Rate of The Workers**
In this study, worker characteristics include weight and duration work-time. The sample in this study were 18 mechanic in Workshop X Tembalang Semarang.

Based on data on worker weight and worker height, workers’ body surface area and respiration rate can be calculated using the following formula.

**A. The Workers’ Surface Area**

$$\text{BSA Human} = \frac{W \cdot h}{3600}$$

**Description:**

BSA: Body surface area (m²)

W : Weight (kg)

h : Height (cm)

**B. Workers’ Respiratory Rate**

$$\text{BR} = \frac{5.3(\ln W) - 6.9}{24}$$

**Description:**

BR : Breathing rate (m³/h)

W : Weight (kg)

Based on Table 2, it was known that the highest body weight of workers in the Workshop X Tembalang Semarang was 74 kg, while the lowest weight was 45 kg. The length of work in a day was all 8 hours and height uses the average value of Indonesian adult male height which was 160 cm.

From the calculation of the body surface area and the respiratory rate of workers it was known that the average body surface area (BSA) of workers was 1.60 m² and the average respiration rate of workers was 0.60 m³/hour.

3. Benzene Concentration

The measurement of benzene concentration at two points in Workshop X showed that the measurement results at location 1 were 0.28 ppm and at location 2 was 0.19 ppm.

**Table 3. Benzene Concentration**

<table>
<thead>
<tr>
<th>Location</th>
<th>Benzene Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location 1</td>
<td>0.28</td>
</tr>
<tr>
<td>Location 2</td>
<td>0.19</td>
</tr>
</tbody>
</table>

The results of measurements, it was known that benzene concentration in Workshop X Tembalang Semarang Location 1 was 0.28 ppm and Location 2 was 0.19 ppm. Based on the Minister of Manpower and Transmigration Regulation Number 13 in 2011 concerning the threshold value of physical factors and chemical factors in the workplace for a concentration of Benzene of 0.5 ppm so that the concentration of benzene in this workplace was still below the NAB. However, the concentration of benzene was above the Minimum risk Level (MRL), the level of benzene inhalation exposure determined by ATSDR, for acute exposure (≤14 days) = 0.009 ppm, moderate exposure (15-364 days) = 0.006 ppm, and chronic exposure (≥365 days) = 0.003 ppm.

4. Animal Km and Human Km

A. Animal Km

$$\text{AnimalKm} = \frac{W_{\text{Animal}}}{\text{BSA}_{\text{Animal}}}$$
Table 4. Animal Calculation Results Km on Animal Experiments

<table>
<thead>
<tr>
<th>Animal Experiments (White Mice)</th>
<th>Animal Km</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5,81420952</td>
</tr>
<tr>
<td>2</td>
<td>5,81420952</td>
</tr>
<tr>
<td>3</td>
<td>5,82102947</td>
</tr>
<tr>
<td>4</td>
<td>5,82102947</td>
</tr>
<tr>
<td>5</td>
<td>5,80052067</td>
</tr>
<tr>
<td>6</td>
<td>5,81420952</td>
</tr>
<tr>
<td>Rata-rata</td>
<td>5.81</td>
</tr>
</tbody>
</table>

The Animal Km calculation shown in table 4. Average Animal Km in animal experiments white rats were 5.81.

B. Human Km

\[
Human\text{Km} = \frac{W_{\text{Human}}}{BSA_{\text{Human}}}
\]

Description:

Human Km: Km factor in Worker

W : Worker weight

BSA : Body Surface Area worker

Table 5. Human Km Calculation for Workers in Workshop X

<table>
<thead>
<tr>
<th>Pekerja</th>
<th>Human KM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33,54</td>
</tr>
<tr>
<td>2</td>
<td>34,20</td>
</tr>
<tr>
<td>3</td>
<td>32,51</td>
</tr>
<tr>
<td>4</td>
<td>39,11</td>
</tr>
<tr>
<td>5</td>
<td>36,74</td>
</tr>
<tr>
<td>6</td>
<td>40,80</td>
</tr>
<tr>
<td>7</td>
<td>33,54</td>
</tr>
<tr>
<td>8</td>
<td>36,43</td>
</tr>
<tr>
<td>9</td>
<td>38,82</td>
</tr>
<tr>
<td>10</td>
<td>39,40</td>
</tr>
<tr>
<td>11</td>
<td>33,87</td>
</tr>
<tr>
<td>12</td>
<td>31,81</td>
</tr>
<tr>
<td>13</td>
<td>32,17</td>
</tr>
<tr>
<td>14</td>
<td>36,74</td>
</tr>
<tr>
<td>15</td>
<td>42,42</td>
</tr>
<tr>
<td>16</td>
<td>32,51</td>
</tr>
<tr>
<td>17</td>
<td>36,43</td>
</tr>
<tr>
<td>18</td>
<td>39,68</td>
</tr>
<tr>
<td>Rata-rata</td>
<td>36,15</td>
</tr>
</tbody>
</table>

The Human Km calculation shown in table 5. Average of Human Km workers at Workshop X Tembalang Semarang was 36,15.

5. NOAEL

NOAEL was the highest dose of a substance in statistical or biological chronic or subchronic toxicity studies that do not show detrimental effects on test animals. Toxicology testing can be used to calculate No Observed Adverse Effect Level (NOAEL) and was useful for clinical trials. To determine the safe limit of concentration of a chemical begins with the toxicity test No Observed Adverse Effect Level (NOAEL).

Swaen (2010) states that benzene NOAEL was 3.0 mg/m³ (0.022 mg/kg).

\[
NOAEL_{\text{benzene}} = \frac{3 \times 0.00013 \times 8}{0.1405}
\]

\[
= 0.022 \text{ mg / kg}
\]
6. **Safe Human Dose**

\[ RfC = \frac{NOAEL}{\frac{AnimalKm}{HumanKm}} \]

Description:

RfC: Safe human dose (m/kg)
Animal Km : Km factor in animals
Human Km : Factor Km in Human

Based on these equations, the calculation of RfC obtained was:

\[ RfC = 0.003 \text{ mg/kg} \]

7. **Safe Limit of Benzene Concentration**

Determination of safe limits of benzene concentration in Workshop X Tembalang Semarang uses formulas (William, 1985; Soemirat, 2003; Davwas, 1991) the following:

\[ C_{safe} = \frac{(SHD)(W)}{(\delta)(BR)(t)} \text{ mg/m}^3 \]

To convert units of mg/m³ to ppm:

\[ C_{safe} = \frac{mg}{m^3} \times 24.5 \text{ ppm} \]

Description:

C safe : safe concentration in the air for workers (mg/m³)
RfC: Safe Human Dose (mg/kg)
W : Weight (kg)
δ :% of substances absorbed by the lung
BR : Human respiratory rate (m³/hour)
t : Duration of working time (hours)
MW : Molecular Weight

Based on the above equation, the calculation results of the concentration of safe concentration of benzene in Workshop X was:

\[ C_{man} = \frac{(0.003)(58.56)}{(50\%)(0.60)(8)} = 0.073 \text{ mg/m}^3 \]

\[ C_{man} = \frac{0.073 \times 24.45}{78.11} = 0.023 \text{ ppm} \]

The calculation of the concentration of safe levels of benzene in the air for workers above can be used to predict the concentration of toxins in the air a safe work environment for workers and to be compared with the NAB established by various institutions either by Indonesian statute, National Standardization Agency, ACGIH, NIOSH and OSHA.

**Discussion**

Benzene in a motorbike repair shop environment needs to be identified to determine the level of exposure that can cause a negative effect to the mechanics. This study measured 2 locations which were considered to have the most exposure to benzene from exhaust gases from motorcycles, Locations 1 and 2 in Workshop X Tembalang Semarang. The results of the measurement of benzene concentrations in these two locations differ because of several things such as the number of repaired motors and the levels of benzene produced by vehicles’ combustion. The more the number of motors was repaired, the higher the exhaust gas from the exhaust of the motor, the higher the concentration of benzene in that location.

Exposure time was the total work time per day. From the research conducted, it was known that the mechanic’s working hours in the motorcycle workshop are 8 hours per day. This result was in accordance with normal working hours according to Labor Law No. 13 in 2003 which states normally, the length of work permitted to each worker was no more than 8 hours/day.

The concentration of benzene exposure in Tembalang X Workshop Semarang was influenced by several factors, but one of the most influential was the condition of the air at the work site (benzene concentration in the air). From the results of the calculation of the safe concentration of benzene, the value of safe C was 0.073 mg/m³ or 0.023 ppm.
What can be done to reduce exposure to benzene in workers was the installation of exhaust fans, the use of PPE such as masks, especially during the process of repairing the motorbike. Another approach was taken by eating foods rich in detoxification enzymes, especially GSH which was usually found in most vegetables such as asparagus, spinach, broccoli, garlic, and onions.

Prevention of benzene exposure requires an active role from both mechanics and workshop owners. It can be done to reduce levels of benzene exposure in the work environment by installing an exhaust fan as a vent in the workspace. Ventilation was the process of providing clean outdoor air which was naturally exchanged with indoor air. The main function of ventilation as an exchange of outside air with the room was also intended to control the air temperature so that it wasn’t hot and stuffy so that it increases the intake of benzene exposure. According to Minister of Health Regulation RI 1077/MENKES/PER/2011 concerning guidelines for air sanitation in a well ventilated room was to meet the criteria for extensive ventilation >10% of the floor area at the workplace.

Conflicts of Interest: All of Authors have no conflicts of interest to declare.

Source of Funding: This article “Determination of Safe Level of Benzene Concentration in Mechanics Workshop “X” Tembalang Semarang” was funded by the Author.

Ethical Clearance: The study was approved by the institutional Ethical Board of Faculty of Public Health, Airlangga University.

All of subjects were fully informed about the procedures and objectives of the study then each subject signed an informed consent form.

Conclusion

The results of measuring the concentration of benzene in Workshop X in Location 1 was 0.28 ppm and Location 2 was 0.19 ppm. Based on Minister of Manpower and Transmigration Regulation Number 13 in 2011, the threshold value of benzene was 0.5 ppm. From the results of measurements in this study when compared with the threshold value then it was still below the set threshold value.

Based on the minimum risk level (MRL) of ATSDR 2007, the concentration of benzene every day that can cause acute effects was 0.009 ppm and that can cause chronic effects was 0.003 ppm. From these standards, it can be seen that the concentration of benzene in this workplace has the potential to have a negative impact on the health of mechanics.

Control measures are needed to minimize the risk of health problems that can be experienced by workers due to benzene exposure. The recommendation given to Semarang Tembalang X Workshop was to periodically monitor benzene levels in the air, use personal protective equipment properly by mechanics and if necessary a health check-up can be done at Workshop X Tembalang Semarang.

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Andrographolide and its Attributes Against Triple-Negative Breast Cancer: A Review of the Literature

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Abstract

Breast Cancer is associated with high incidents and mortality rates and considered to be the second most frequent type of cancer among women worldwide. One of its invasive modalities is the Triple-Negative subtype. Lacking the expression of ER/PR and HER2 contributes to its severity. Current treatment strategies for TNBC diagnosed patients are believed to be hazardous and do not improve the disease-free survival (DFS) neither the overall survival (OS). Thus, scientist and researchers are seeking alternative remedies that have little or no consequences on the patients. Andrographis paniculata with its remarkable active constituent Andrographolide are attracting the world’s attention due to its pharmacological activities against metabolic maladies especially hyperlipidemia, obesity, and diabetes as well as its attribute as an anti-cancer compound. Here, we review the effect of Andrographolide on Triple-Negative Breast Cancer.

Keywords: Andrographolide; Breast Cancer; Triple-Negative Breast Cancer; Alternative and Complementary Treatment; Andrographis paniculata.

Introduction

Estrogen, progesterone, and HER2 are the hallmark for the presence of a Breast Tumor, it serves by mimicking the receptors found in the normal breast tissues and sends signals to cells permitting its growth. Nearly 50% of Breast tumors express ER, PR, and HER2 but not in case of the TNBC, in which the expression of the biomarkers is idle 26. TNBC severity exceeds the positive BC. It has a high risk of re-occurrence and death 20 and can metastasize visceroally to other organs (lymph nodes, bilateral lungs, and the liver) 26. Metastatic TNBC has a low life expectancy rate and it does not exceed 12 months in spite of the response to chemotherapy 8. Annually, from 1.3 million Breast Cancer patients 15-20% will be identified as Triple-Negative Breast Cancer subtype 2. The survival rate of TNBC varies depending on the stage of the disease. Stage 0 and 1 have a 100% chance of survival, stage 2 and 3 have 93%, 72% survival rate respectively. Metastatic TNBC patients have 22% 9.

A. paniculata is part of Acanthaceae family, a herbal plant natively found in Sri Lanka and India and it is also found in the southeast of Asian, America, and China 16. A. paniculata has been used traditionally in treating various types of diseases like fever, dysentery, diabetes, and malaria 12. Andrographolide is the major active constituent of in A. paniculata, it belongs to the labdane diterpenoid chemical class. Andrographolide possess many pharmacological properties, it has anti-inflammatory, antihyperglycemic, antimicrobial, antiparasitic and anticancer effects 12.

Current treatment strategies for Triple-Negative Breast Cancer (TNBC)

Chemotherapy (CT) remains the standard strategy for treating TNBC 13. Despite the fact that TNBC is aggressive in nature, yet, it shows a higher vulnerability to Chemotherapy than any other types of Breast Cancers 25. Neoadjuvant systemic treatment (NST) chemotherapy is used for those who are at the early stages of TNBC and willing to undergo BCS 17. frequently used neoadjuvant agents are anthracycline alone or in combination with taxane (anthracycline-taxane CT) 23. In adjuvant settings, anthracycline-taxane regiments show small but important improvement in disease-free survival (DFS) 6.

Breast Conservative surgery is advised for patients with early stage of TNBC alongside adjuvant RT 10. One of its advantages is the low rate of local recurrence, but, it does not imply on all types of Breast Cancer. 25. Mastectomy is wanting to BC because of the better outcomes of BCS, in terms of overall and disease-free
survival rates.

Radiotherapy, as reported by Langlands et al., has little influence on TNBC and can withstand it. Although most of TNBC tumors are associated with BRCA1/2 mutations which means it carries DNA repair defects, however, it showed no significant sensitivity to RT. A contrasting study suggested otherwise, in which RT can enhance survival outcomes if implemented with surgery and it could provide ideal tumor management with trivial side effects.

Targeted therapies have shown its efficacy in targeting the tumor’s cell cycle, gene defects, and growth factors. Affecting cell cycle regulators (CDK4/6) can be tricky in the case of TNBCs since not all express cell cycle regulators. But, a different study tested palbociclib (CDK inhibitor) on Luminal Androgen Receptor (LAR) a subtype of TNBC and exhibited high sensitivity towards it (in vitro and in vivo) 3.

Another potential targeted therapy candidate is the tumor suppressor gene (p53) through checkpoint kinase 1 (CHK1). Since the majority of TNBCs are Basal-Like tumors that carry gene defects in P53 24, Bryant et al., reported that TNBC cell line can be inhibited through arresting cell cycle at the S and G2/M phases mainly by inhibiting CHK1 through the activation of P53 11.

Targeting growth factors such as FGFR, EGFGR, and VEGFR can beneficial in eliminating TNBC since they are the causative agent for the overgrowth of the tumor 4. Drugs such as cetuximab (anti-EGFR antibody), PD173074 (anti-FGFR inhibitor) and bevacizumab as a VEGFR inhibitor currently being tested on TNBC 21.

**Andrographis paniculata**

*Andrographis paniculata* is part of Acanthaceae family, a herbal plant natively found in Sri Lanka and India and it is also found in the southeast of Asian, America, and China. *A. paniculata* is a herbal grows annually and extend about half to a meter above the soil, it is known for its bitter taste hence the name King of Bitter 16. *A. paniculata* has been used traditionally to cure diseases like fever, dysentery, diabetes, and malaria. In Asia, the roots and the aerial parts have been used to treat inflammation, pyrexia, intermittent fevers and stomachaches. The essence of leaves is used to treat fever, infectious diseases, colic pain and diarrhea, the aerial part of the plant is used in Malaysia to treat snakebites and malaria 16.

The active compounds are distributed throughout the parts of the plant for instance terpenoids. The most isolated terpenoid from the plant is Andrographolide in terms of quantity and occurrence. Andrographolide after it is isolated, it appears crystalline and colorless and has a bitter taste. Apart from Andrographolide, neoAndrographolide and deoxyAndrographolide are also common terpenoids isolated from the aerial parts and the roots.

Flavonoids such as Flavone-1, 2’methylether, 7-O-methylwogonin and flavone-1, 2’-O-glucoside are found in the whole body, roots and the aerials of *A. paniculata*.

**Andrographolide**

Andrographolide is the major active constituent found in *A. paniculata*, it belongs to the labdane diterpenoid chemical class. Andrographolide is the causative agents for the extremely bitter taste of *A. paniculata*. Andrographolide must be harvested after cultivating the plant for 110 days or just before the flowering of the plant. It can be found in several parts of the plant including the roots, leaves, stem, the aerial parts or in the whole plant. As for the process of isolation, it can be achieved through extraction by solvents like ethanol (EtOH), methanol (MeOH), acetone, dichloromethane and chloroform (CHCl3). *A. paniculata* synthesizes Andrographolide through two pathways, the mevalonic acid pathway or through deoxyxylulose pathway (DXP). Andrographolide possess many pharmacological properties, it has anti-inflammatory, antihyperglycemic, antimicrobial, antiparasitic and anticancer effects.

Anti-Cancer Effect of Andrographolide on TNBC cell lines

Anti-proliferative and viability effect

Several studies suggested that among all TNBC cell lines, MDA-MB-231 was the most susceptible type of TNBC cell line towards Andrographolide. Weber et al., proposed that 39.6μg/mL of Andrographolide was capable of eliminating 50% of cells, meanwhile, a higher dose of Andrographolide (77.6μg/mL) was able to inhibit MDA-MB-468 population. A different report was done by M Banerjee et al, where they implemented Andrographolide on multiple BC cell lines (MCF-7, MDA-MB-231, and T-47D), and used MCF-10A (normal breast tissue cell line) to examine its effect on the normal breast epithelia. The pattern of exposure
to Andrographolide was in both time and concentration-dependent manners. MDA-MB-231 cell line was found to be the most vulnerable amongst the other cell lines, and the exposure to the treatment introduced inhibitory effect on both viability and propagation of cells. The IC\textsubscript{50} was estimated to be 51.98μM at 24 hrs. and 30.28μM at 48 hrs. Worth mentioning that these values did not cause any effect on the MCF-10A, however, to introduce an effect the needed concentrations must be 3-5 folds higher. Similar outcomes were reported by Peng et al., in which approximately 50μM of Andrographolide eliminated MDA-MB-231 population.  

The induction of cell cycle arrest  

Andrographolide has the ability to induce cell cycle arrest as reported by several studies. Increase in cellular population was reported by M Banerjee et al., at S phase and a decrease occurred at G1/G0 accompanied by the formation of Reactive Oxygen Species (ROS), the increase in ROS was proportional to a longer time of exposure and might indicate that the inhibition was due to ROS accumulation. Different results were suggested by another study, the increase in population happened at the G1 and the decrease was noticed at the S phase. Downregulation in the levels of P27 (G1 phase inhibitor) and CDK6 were detected, and no accumulation in ROS was observed. While in a study done by Alqouqa et al., the arrest occurred at G\textsubscript{2}/M phase, followed by downregulation of Cyclin D1, CDK4, Cyclin E and P-cdc2, and upregulation of P21, Cyclin B1 and Waf1/Cip (cell cycle regulators proteins).  

The introduction of cellular and nuclear morphological modifications  

Nuclear morphological modifications were described as well, nuclear pyknosis, chromatin condensation, irregular edges and body fragments formation occurred during the time of exposure.  

Mitochondrial and apoptotic effects  

Release of cytosolic cytochrome c from the mitochondria of MDA-MB-231 was recorded. The release might be caused by the changes in the ratio of apoptotic and anti-apoptotic proteins (Bax/Bcl-2), whereas Andrographolide elevated their expression. In the same fashion, Peng et al. detected the translocation of cytochrome c from the intermembrane to the cytosol. Bcl-2, Bax and cleaved caspase-3/9 were spotted after 24 hrs. dosage of Andrographolide. Caspase-3/9 activation correlates with the finding of M Banerjee et al.  

Conclusion and Future Directions  

Breast Cancer still remains a threat to women around the world. Despite technological advancement and the increase in awareness, many patients are not transitioning from ill to healthy. The undeniable fact that the current treatments (Radiation Therapy (RT), Chemotherapy (CT) and Adjuvant Endocrine) have proved its effectiveness. But, its complications on the patients are treacherous and the medical community is due to overcome it. Andrographolide affects TNBC cells through multiple pathways such as the arresting of the cell cycle, inducement of cellular and nuclear morphological changes, upregulation apoptotic and anti-apoptotic proteins (Bax/Bcl-2). Involving Andrographolide in animal or human trials could elucidate its potential as an anti-cancer agent and provide knowledge and assessment for the future references.  

Acknowledgment: The author would like to acknowledge the aid and guidance of Dr. Nik Norliza and Dr. Noraishin Hanifa and all the support provided by the University Sains Malaysia.  

List of Symbols and Abbreviations  

**Abbreviation**  

BAX: BCL2-Associated X protein  

Bcl: B-Cell Lymphoma  

BCS: Breast-Conserving Surgery  

BL1: Basal-Like 1  

BL2: Basal-Like 2  

BRCA: BReast CAncer gene  

CDC: Cell Division Control protein.  

CDK: Cyclin-Dependent Kinase  

CHK: Checkpoint Kinase  

CT: Chemotherapy  

DFS: Disease-Free Survival  

DNA: DeoxyriboNucleic Acid
EGFR: The Epidermal Growth Factor Receptor
ER: Estrogen Receptor
ET: Endocrine Therapy
HER2: Human Epidermal Growth Factor Receptor
HR: Hormone-Receptor
Hr: Hour
LAR: Luminal Androgen Receptor
M: Molar
MCF7: Michigan cancer foundation-7
ml: Milliliter
mm: Millimeter
OS: Overall Survival
PR: Progesterone Receptor
RT: Radiotherapy

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Ethical Procedure: The research meets all applicable standards with regard to the ethics of experimentation and research integrity, and the following is being certified/declared true.

References


Cortisol And Psychological Factors in Etiology of Lichen Planus

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Abstract

Background and Objectives: Lichen planus is a common chronic inflammatory disease of oral mucosa and skin, whose exact pathogenic mechanisms have not been understood. Cortisol, has been used as an indicator in various psychological evaluation studies. Salivary cortisol measurement is an indicator of free cortisol in human serum and provides noninvasive and easy technique.

Aims, evaluation of cortisol levels and psycho-immunity profile in lichen planus patients was done. Using case-control method.

Materials and Method: 145 participants were admitted which had been done at dermatological outpatient clinic at Al-Yarmook Teaching Hospital. 32 patients were clinically proven cases of LP, 113 subjects along with age and sex-matched healthy controls. DASS Score questionnaire was administered to evaluate the psychiatric status (depression, anxiety and stress), Saliva samples were collected, and analyzed for cortisol level by using ELISA in study group and 46 of control group. The serum test were examined in 32 for each group using enhanced lanthanide fluoroimmunoassay technique for cortisol.

Results: The mean serum and Salivary cortisol level of the LP group showed a very highly significant difference (p=0.001) from the controls in multivariate statistic. The mean of DASS scores and the depression derangement specifically showed a very highly significant difference (p=0.001) from the controls. The stress and anxiety derangement showed high difference but it failed to reach to significant difference from the controls. There is an important association between serum and salivary cortisol concentration.

Conclusion: the cortisol and psychiatric factors play a vital role in the pathogenesis of LP and saliva cortisol could be a possible indicator instead of serum cortisol

Key Words: Lichen Planus, Cortisol, DASS, Stress, Depression, Anxiety

Introduction

Lichen planus is a chronic inflammatory mucocutaneous disorder, which can manifest in the oral mucosa. Oral lichen planus affects 0.1-4% of various populations. The disease usually affects middle-aged to elderly females. Currently the precise cause of lichen planus is unclear. Stress, depression and anxiety are the most common psychological factors, which causes alteration of the body tissues in general and oral in specific⁴. The world health organization classified OLP into several clinical forms: Reticular, papular, plaque – like, atrophic, erosive, ulcerative and bullous. The most often affected areas are the posterior buccal mucosa and tongue, the non-erosive form is often asymptomatic. The erosive forms are commonly sensitive or painful and affect the quality of life⁵.

Stress alters the regulation of both sympathetic and parasympathetic branches of autonomic nervous system, with consequential alteration in hypothalamic control of endocrine response controlled by the pituitary gland, autonomic activation and elevation of hormones, including those produced by hypothemic-pituitary – adrenal axis, play pivotal roles in regulation immune surveillance mechanism, including production of
Cytokines that control the inflammatory process (3).

Cortisol is a 21-carbon glucocorticoid secreted by the adrenal cortex that regulates carbohydrate, protein, fat and water metabolism, maintains vascular reactivity, affects the sensitivity of the nervous system, regulates blood cell numbers, and affects the human stress response (4). Cortisol levels can be measured in urine, and saliva. This approach has several methodological advantages, including the noninvasive collection of samples, reduced stress and repeatable sampling unlike blood. Cortisol in saliva is a useful index for investigations of chronic and acute stress reflected in HPA axis activity (5).

The objectives of this study are: Estimate serum and unbounded salivary cortisol level in Lichen Planus patient, evaluate the psychological disorders (depression, anxiety and stress) levels using Depression Anxiety Stress scale in Lichen Planus patient, and evaluate correlation between serum and salivary unbounded cortisol in Lichen Planus patient.

Patient selections: A total of thirty-two patient with Lichen Planus seen in the dermatological clinic at Al Yarmmok Hospital. Questionnaires: is including name, complete history, Depression Anxiety Stress Scales - DASS21 (the DASS is a set of three self-report scales designed to measure the negative emotional states of depression, anxiety, and stress), Salivary cortisol: A-Reagents supplied in unbounded salivary cortisol kit, and Serum cortisol kit: Vidas cortisol kit: Is used to determine cortisol on serum.

Method

DASS questionnaires: All case-control subjects are asked to use 4-point severity/frequency scales to rate the extent to which they have experienced each state over the past week.

Salivary and Serological laboratory tests: Collecting Saliva: The participants gave 5 minutes to collect saliva in the plain tube. The time of collection was between (9:30 am-12:00 pm) to pool saliva and refrigerate samples within 30 minutes and freeze at or below -20°C within 4 hours after collection. The Serological measurements were done in Teaching Laboratories at Al Yarmook Hospital for each group.

Statistical Analysis: Data were translated into a computerized database structure. A multiple linear regression model was used to study the net and independent effect of a set of explanatory variable on a quantitative outcome (dependent) variable like salivary cortisol concentration. The model provides the following parameters: P (model), Unstandardized partial regression coefficient, P for regression coefficient, and R². A multiple logistic regression model was used to assess the net role of a set of explanatory variables in predicting Lichen Planus cases differentiating them from healthy controls, the model provides the following parameters: P-value for the model, The overall predictive power, Adjusted OR, and P-value for OR.

Cases Distribution: 32 patient included in the study were distributed into 21 females and 11 males, the female to male ratio was 1.9:1. While the control group consisted of 113 divided into 57 females and 56 males. The female ratio was almost 1:1. For the Lichen Planus group their ages were ranged between (16-60) with a mean ±SD 41.6 ±13.2 years. Compare to (19-67) years in control groups with a mean± SD are 41.8 ±11.4 years. The differences failed to reach the level of statistical significant 0.1{NS}. The age difference between study groups is not an important confounder.

<table>
<thead>
<tr>
<th>parameters</th>
<th>group</th>
<th>range</th>
<th>mean</th>
<th>SD</th>
<th>SE</th>
<th>n</th>
<th>p(t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum cortisol</td>
<td>Control</td>
<td>(1.8-192.9)</td>
<td>171.4</td>
<td>60.9</td>
<td>11.13</td>
<td>30</td>
<td>0.23{NS}</td>
</tr>
<tr>
<td></td>
<td>Cases</td>
<td>(40.1-194)</td>
<td>194.4</td>
<td>85.1</td>
<td>15.04</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cohen’s effect size of being a case compared to control =0.43</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salivary unbounded cortisol</td>
<td>Control</td>
<td>(1.8-192.9)</td>
<td>171.4</td>
<td>60.9</td>
<td>11.13</td>
<td>30</td>
<td>0.82{NS}</td>
</tr>
<tr>
<td></td>
<td>Cases</td>
<td>(40.1-194)</td>
<td>194.4</td>
<td>85.1</td>
<td>15.04</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cohen’s effect size of being a case compared to control =0.34</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1 case-control difference in Serum and Salivary cortisol, the differences failed to reach the level of statistical significant 0.1{NS}
Asses the magnitude the case-control difference in DASS scale

The anxiety Score (/100) showed obviously higher 7 in comparison with healthy controls. The effect of the disease on the anxiety Score was moderate (Cohen’s =0.38).

The depression score (/100) showed obviously higher 11.1 in comparison with healthy controls (27.9). The effect of the disease on the depression score was moderate (Cohen’s =0.58).

The stress Score (/100) showed obviously higher 6.5 in comparison with healthy controls (42.6). The effect of the disease on the stress Score was moderate (Cohen’s =0.31).

The DAS Score (/100) showed obviously higher 8.2 in comparison with healthy controls (33.3), the effect of the disease on the depression score was moderate (Cohen’s =0.48).

Table 2 Asses the magnitude the case-control difference in DASS scale

<table>
<thead>
<tr>
<th>DASS scale</th>
<th>range</th>
<th>mean</th>
<th>SD</th>
<th>SE</th>
<th>n</th>
<th>p(t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anxiety Score (/100)</td>
<td>(4.8 - 71.4)</td>
<td>36.4</td>
<td>17</td>
<td>3.01</td>
<td>32</td>
<td>0.06[NS]</td>
</tr>
<tr>
<td>Control</td>
<td>(0 - 81)</td>
<td>29.4</td>
<td>18.7</td>
<td>1.76</td>
<td>113</td>
<td></td>
</tr>
<tr>
<td>Cohen’s effect size of being a case compared to control 0.38</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depression Score (/100)</td>
<td>(0 - 85.7)</td>
<td>39</td>
<td>21.3</td>
<td>3.76</td>
<td>32</td>
<td>0.004</td>
</tr>
<tr>
<td>Control</td>
<td>(4.8 - 85.7)</td>
<td>27.9</td>
<td>18.5</td>
<td>1.74</td>
<td>113</td>
<td></td>
</tr>
<tr>
<td>Cohen’s effect size of being a case compared to control = 0.58</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stress Score (/100)</td>
<td>(14.3 - 90.5)</td>
<td>49.1</td>
<td>17.5</td>
<td>3.1</td>
<td>32</td>
<td>0.13[NS]</td>
</tr>
<tr>
<td>Control</td>
<td>(4.8 - 90.5)</td>
<td>42.6</td>
<td>22.2</td>
<td>2.09</td>
<td>113</td>
<td></td>
</tr>
<tr>
<td>Cohen’s effect size of being a case compared to control = 0.31</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAS Score (/100)</td>
<td>(12.7 - 76.2)</td>
<td>41.5</td>
<td>16.3</td>
<td>2.88</td>
<td>32</td>
<td>0.017</td>
</tr>
<tr>
<td>Control</td>
<td>(6.3 - 76.2)</td>
<td>33.3</td>
<td>17.3</td>
<td>1.63</td>
<td>113</td>
<td></td>
</tr>
<tr>
<td>Cohen’s effect size of being a case compared to control = 0.48</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Table 3 a multiple logistic regression model was used. After adjusting for the confounding effect of age, gender, magnitude of psychiatric derangement and serum immunoglobulins, it was found that serum cortisol concentration was significantly higher among cases with Lichen Planus. Depression score was the most affected among the 3 measures of psychiatric derangement.

<table>
<thead>
<tr>
<th></th>
<th>OR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anxiety score (/100)</td>
<td>1.04</td>
<td>0.28[NS]</td>
</tr>
<tr>
<td>Depression score (/100)</td>
<td>1.05</td>
<td>0.079</td>
</tr>
<tr>
<td>Stress score (/100)</td>
<td>1.0</td>
<td>0.92[NS]</td>
</tr>
<tr>
<td>Serum Cortisol</td>
<td>1.02</td>
<td>0.023</td>
</tr>
<tr>
<td>Age in years</td>
<td>0.97</td>
<td>0.34[NS]</td>
</tr>
<tr>
<td>female gender compared to male</td>
<td>7.07</td>
<td>0.022</td>
</tr>
</tbody>
</table>

P (Model) < 0.001

Overall prediction accuracy = 82.3%
Table 4 A multiple logistic regression model was used. After adjusting for the confounding effect of age, gender and magnitude of psychiatric derangement. It was found that salivary cortisol concentration was significantly higher among cases with Lichen Planus.

<table>
<thead>
<tr>
<th></th>
<th>OR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anxiety score (/100)</td>
<td>1.02</td>
<td>0.39[NS]</td>
</tr>
<tr>
<td>Depression score (/100)</td>
<td>1.01</td>
<td>0.71[NS]</td>
</tr>
<tr>
<td>Stress score (/100)</td>
<td>1.04</td>
<td>0.15[NS]</td>
</tr>
<tr>
<td>Age in years</td>
<td>0.99</td>
<td>0.76[NS]</td>
</tr>
<tr>
<td>female gender compared to Male</td>
<td>3.96</td>
<td>0.025</td>
</tr>
<tr>
<td>Salivary cortisol</td>
<td>56.8</td>
<td>0.053</td>
</tr>
</tbody>
</table>

P (Model) < 0.001

Overall prediction accuracy = 77.2%

Table 5 The mean salivary cortisol was lowest (0.079) among cases with lowest concentration of serum cortisol (first tertile) and increase to reach its highest mean concentration (119) among cases with highest concentration of serum cortisol (third tertile). The differences observed in mean salivary cortisol were statistically significant. There was a moderately strong positive (direct) linear correlation between serum and salivary cortisol concentration (r=0.54 , P=0.001).

The mean salivary cortisol by 3 serum cortisol ordered categories (tertiles).

<table>
<thead>
<tr>
<th>Serum Cortisol-tertile</th>
<th>First tertile (&lt; 82.6)</th>
<th>Second tertile (82.6 - 118.9)</th>
<th>Third tertile (119.0+)</th>
<th>P (ANOVA trend)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salivary cortisol</td>
<td></td>
<td></td>
<td></td>
<td>0.048</td>
</tr>
<tr>
<td>Range</td>
<td>(0.012 - 0.319)</td>
<td>(0.036 - 0.255)</td>
<td>(0.008 - 0.629)</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.079</td>
<td>0.137</td>
<td>0.209</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>0.1</td>
<td>0.081</td>
<td>0.196</td>
<td></td>
</tr>
<tr>
<td>SE</td>
<td>0.0353</td>
<td>0.0243</td>
<td>0.0544</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>8</td>
<td>11</td>
<td>13</td>
<td></td>
</tr>
</tbody>
</table>

Table 6 a multiple linear regression model was used to assess the net and independent effect of a set of explanatory variables on the magnitude of salivary cortisol concentration. Only serum cortisol had a statistically significant association with salivary cortisol concentration after adjusting for age and gender, the 3 psychiatric scores and group membership (Lichen Planus Vs controls).
Conclusion: There is an important association between serum and salivary cortisol concentration.

<table>
<thead>
<tr>
<th>Partial regression coefficient</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Constant)</td>
<td>-0.25</td>
</tr>
<tr>
<td>Serum Cortisol</td>
<td>0.001</td>
</tr>
<tr>
<td>Age in years</td>
<td>0.002</td>
</tr>
<tr>
<td>female gender compared to male</td>
<td>0.008</td>
</tr>
<tr>
<td>Lichen Planus compared to healthy controls</td>
<td>0.028</td>
</tr>
<tr>
<td>Anxiety score (/100)</td>
<td>-0.002</td>
</tr>
<tr>
<td>Depression score (/100)</td>
<td>-0.001</td>
</tr>
<tr>
<td>Stress score (/100)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

$P$ (Model) = 0.06

R² = 0.

Lichen Planus (LP) is a chronic inflammatory, autoimmune disease, affecting a wide variety of sites, including skin and mucous membranes. (6).

Anxiety, stress, depression and other psychological aspects of humans and their association with disease status were the domain of extensive research. Chronic conditions like Lichen Planus deserve special attention in this respect. (7).

Cortisol hormone is well established in medical literature as the stress hormone. Its direct relation to psychology is a known fact (8). The current study is the first of its kind in Iraq, its importance was reflected by exploring the association between psychology and cortisol both serum and salivary.

Three important domains of psychological abnormality in human were studied in the present work: stress, anxiety and depression.

Measurement of depression and its association with Lichen Planus was done by several scales. Wu XH, 2011 used self-rating depression scale (SDS), Hamilton depression scale (HAMD) (9).

Measurement of anxiety and its association with Lichen Planus was done by several scales. Two published articles by Maryam Rabiei (10), and Koray M, 2003 used Spielberger’s State-Trait Anxiety Inventory. EL Tawil M use Taylor manifest anxiety (11).

Measurement of stress and its association with Lichen Planus was done by several scales and indices in published articles. Farhad Mollashahi Leila, used Stress Life Event Questionnaires (12).

The current study used an updated psychology scale, which can measure the magnitude of anxiety, stress and depression as an independent entity with only 7 questions per domain.

DASS as a measure of psychology and its association with Lichen Planus or Cortisol Lichen Plan us Cortisol was used to a great extent in recently published articles. Singh R used DASS in measuring stress and its effect on cortisol level in medical students (13). Madhumita Premkumar also used DASS in measuring changes in mood and assessed the correlation with cortisol (14).

The current study showed a statistically significant positive association between psychiatric derangement and Lichen Planus. The mean DASS score was significantly higher in Lichen Planus cases compared to healthy control group. Depression was the almost affected to Lichen Planus.

The current study showed that the mean of serum and salivary cortisol was obviously higher with LP compared to control group.

A list of published case-control studies reported similar positive association between salivary cortisol and Lichen Planus. Maryam Rabie (10), Carla Girardi (15), Sanjib Kumar Khataniar (16), and Bina Shah (17), measured
salivary cortisol detected by ELISA. They concluded that patients with oral Lichen Planus exhibited greater salivary cortisol than the controls.

The current study showed a strong direct correlation between serum and free unbound salivary cortisol in patients with Lichen Planus.

Published articles agreed with the previous conclusion. Elisabeth Aardal (18) and Vining RF (19), studied the relation between free salivary cortisol and serum cortisol in healthy volunteers. They concluded that the relation between the cortisol concentrations in serum and saliva was positive.

Two published articles showed that salivary cortisol in critically ill patients may be a better laboratory indicator of cortisol levels than serum total cortisol. Rômulo Carvalho compared the performance of salivary and serum total cortisol in patients with severe sepsis (20). Aardal-Eriksson showed that CRH stimulation induced a more pronounced cortisol response in saliva than in serum, having a closer correlation with ACTH hormone over serum cortisol. The study suggested that salivary cortisol measurement may be used as an alternative parameter in dynamic endocrine tests (21).

**Conclusion**

1. The mean of salivary and serum cortisol was higher difference in Lichen Planus than control subjects, the mean DASS and the special depression Derangement also higher in LP patient compared to control subjects (p<0.001).

2. The current study showed a strong direct correlation between serum and free unbound salivary cortisol in patients with lichen planus. Salivary cortisol in critically ill patients may be a better laboratory indicator of cortisol levels than serum total cortisol.

**Suggestion**

1. As the present study is pioneering in Iraq, we recommend that further studies should be carried out with increasing the sample of patients for more accuracy and confirmation of results. Also we recommend for using psychiatric drug along with routine LP treatment to determine the efficacy of drug therapy of psychiatric disorders in lichen planus.

**Ethical Clearance** A consent form has been signed by each participant and the agreement of the ethical committee at the Department of Oral Medicine was taken.

**Primary Founding source** - Self

**Conflict of Interest**: Nil

**References**

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Purification of L-Glutaminase from *Bacillus sp. B12* and study its properties

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Abstract

L-glutaminase is an enzyme that catalyses the conversion L-glutamine to glutamic acid and ammonia. The important application of the L-glutaminase is a chemotherapy agent. In this study a novel strain, *Bacillus B12*, that isolated from Diyala soil was explored for production of extracellular L-glutaminase. The enzyme has been purified 23.9-fold from cell-free extract with 45.4% recovery (specific activity 76.6 U/mg protein). Enzyme has molecular weight of 199 kDa, and pH 8 and stable in pH range 6-9.5. Temperature optimum is 40 °C and completely stable between 25-45 °C. The kinetics studies revealed that the km and Vmax of purified L-glutaminase were estimated in 0.4mmol/L and 0.133mmol/min, respectively. The result showed that, the enzyme was active when incubated with 10 mM of Mn2+, Mg2+, Ca2+ and Na+. Whereas, K+, Co2+, and Ni2+ show no effect. However, Hg2+, Cu2+, Fe2+ and Zn2+ decreased L-glutaminase activity. The 10mM of DEAE, PMSF, and sodium azide did not show a clear effect against the enzyme activity. Higher decrease in enzyme activity was observed by using Cysteine and 2-Mercaptoethanol (30 and 25%, respectively).

Keywords: *Bacillus sp. B12*, L-glutaminase, purification, Ion exchange, pH, and Thermostability.

Introduction

Microbial communities are an important biological component of soil function, and all organisms in an ecosystem rely on the activity of microorganisms for their role in improving soil quality and regulating nutrient availability [1]. Producing extracellular enzymes which are needed to break down organic material for sustainable soil productivity. L-glutaminase plays a major role in the nitrogen metabolism of both prokaryotes and eukaryotes [2]. It is an important enzyme therapy for a variety disease of the lymphatic system especially acute lymphocytic leukemia [3].

The enzyme has also been applied in the treatment of Hodgkin’s disease, lymphosarcoma, and acute myelocytic leukemia [4]. L-glutamine is not an essential amino acid for the animal cell, and the neoplastic cells are not able to induce the synthesis of glutamine synthetize. However, they are dependent on the extracellular level of L-glutamine to protein synthesis [5]. As L-glutamine is withdrawn from plasma by L-glutaminase, this amino acid leads to inhibit the growth in neoplastic cells [6]. There is a great demand to produce chemotherapy, a variety of nutritional and physiochemical factors influences the production of enzymes. For this reason, this enzyme has been developed to use as therapy to tumor cells [7]. Nowadays optimization of different production parameter would enhance the quality and quantity of enzyme production, and turn in turn influence enzyme synthesis and cell yield [8]. The microbial source is very common for L-glutaminase, because of easily cultured, extraction, and purification for the industrial scale production. The most used microorganisms to produce L-glutaminase *E.coli*, *Actinomycetes*, *Pseudomonas sp.*, *Erwinia carotovora sp.* [9]. Because of the continuous need to screen newer strains that capable to produce high yield of L-glutaminase, this paper deals with the purification, kinetics properties, and biochemical characterization of L-glutaminase from *Bacillus B12*.

Method

Bacterial strain, culture condition and enzyme purification

Bacterial strain *Bacillus sp*.B12 (Genbank accession
no.:SAMN12069785) was used. The strain was grown on nutrient agar at 30 °C for 24 h.

**L-glutaminase purification**

All purification steps were carried out at 4°C. *Bacillus sp. B12* L-glutaminase was purified by a modification of the method of Boyd and Philips [10].

**L-glutaminase assay**

The enzyme was assayed by direct nesslerization method according to Imada et.al [11].

Estimation of protein content

The protein concentration was determined using Lawry method [12]. A calibration curve was made by using bovine serum albumin (BSA) as standard.

**Molecular weight determination**

Molecular weight of the purified L-glutaminase was estimated by Sephadx G-100 performed as described above using standard proteins (BSA, trypsin, pepsin, and catalase) and proteins standard curve [13].

**Effect of pH on enzyme activity and stability**

The optimum pH for enzyme activity and stability was determined. The enzyme was incubated for 1 hr at various pH values (4-11).

**Effect of temperature on enzyme activity and stability**

The optimum temperature for enzyme activity and stability were determined. The enzyme was incubated in different temperature values (10°C to 60°C).

**Influence of different effectors on L-glutaminase activity**

The enzyme activity was determined in the presence of different metal salts and other compounds (10mM). Under the standard assay conditions, the enzyme was incubated for 60-min at 35°C with the each effector. The relative activities were determined by considering 100% activity of enzyme in the absence of additives.

**Kinetics parameters**

Kinetic parameters were determined using the hydrolysis the substrate L-glutamine (0-20mmol/L) under standard assay conditions. The Michaelis-Menten (km) constant and maximum velocity (Vmax). The reaction mixture was 0.02M tris-HCL buffer, pH 8.6. The values of Km and Vmax were then determining from line weaver-Burk plot [14].

**Results and Discussion**

**Purification of L-glutaminase and molecular mass estimation**

L-glutaminase from *Bacillus* sp.B12 was purified 23.9 folds, with yield of 45.4 (Table1) after sulfate fractionation (68%). Fast column chromatography was performed through the Ion exchange DEAE-Cellulose followed by Sephadex G-100 (Fig.1.) These results were in the agreement with L-glutaminase activity from *Bacillus* sp. LKG-01 was purified to 49-fold with 25% recovery with specific activity 584.2 U/mg protein [15].

**Table 1. sequential multi-steps process for purification of L-glutaminase from Bacillus sp. B12**

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Volume (ml)</th>
<th>Total protein (mg/ml)</th>
<th>Specific activity (U/mg)</th>
<th>Total activity (U)</th>
<th>Purification Fold</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture supernant</td>
<td>45</td>
<td>3.6</td>
<td>1.1</td>
<td>3.2</td>
<td>162</td>
<td>1</td>
</tr>
<tr>
<td>Ammonium Sulphate (68%)</td>
<td>20</td>
<td>4.5</td>
<td>0.9</td>
<td>3.9</td>
<td>90</td>
<td>1.2</td>
</tr>
<tr>
<td>DEAE cellulose</td>
<td>13</td>
<td>6.2</td>
<td>0.6</td>
<td>10.3</td>
<td>80.6</td>
<td>3.2</td>
</tr>
<tr>
<td>Sephadex G-100</td>
<td>8.0</td>
<td>9.2</td>
<td>0.12</td>
<td>76.6</td>
<td>73.6</td>
<td>23.9</td>
</tr>
</tbody>
</table>
Characterization of purified L-glutaminase

Determination the molecular weight

The molecular homogeneity of purified L-glutaminase revealed a specific activity of 73.6 U/mg, and the molecular weight was estimated as 199 KDa (Fig.1). There has been wide variation in the molecular weight of L-glutaminase produced from different sources, for instance the strain Pseudomonas aurantiaca produced 148 kDa [16]. Also, Elshafei et al (2014) demonstrated that an intracellular L-glutaminase from Penicillium brevicompactum NRC829 showed molecular mass 71kDa [17].

While, the purified L-glutaminase from Pseudomonas aeruginosa 50071 presented the molecular weight 160kDa [18]. Similarly, the partially purified enzyme from Bacillus cereus MTCC 1305 showed an approximate molecular weight 140 kDa [19]. Most of the L-glutaminase are monomers; however, the enzyme of Erwinia carotovora exists in the form dimer with molecular weight 40.2kDa [20].


C: Native molecular mass estimation by gel filtration. Standard markers: Trypsin (23.3 kDa) Pepsin (34.5 kDa), BSA (66.4 kDa), Catalase (57.3 kDa). The molecular weight size marker (b), molecular mass determination for enzyme (b).

Figure 1. L-glutaminase purification and characterization.

Effect of pH and temperature on the activity and stability on the purified L-glutaminase

L-glutaminase is one of the amidases that are generally active and stable at neutral and alkaline pH. Figure 4 revealed that pH. 8 was the optimal for L-glutaminase activity (3U/ml) (Fig.2). In agreement with our results, Elshafei et al [17] reported that, pH. 8.5 to be the optimum pH for amidase activity. Continuously, an optimum pH range of 7.5 to 9 was found for L-glutaminase production from Pseudomonas aeruginosa [21].
The purified L-glutaminase from *Bacillus* sp. B12 was more stable in alkaline pH more than acidic one; it returns more than 75% of the total activity in the pH range 6-10. Our results also demonstrated that L-glutaminase retained about 50% of its activity after storage at pH 11 (Fig.2). Ohshima reported that, pH from 9.5 to 10 seems to be the most suitable pH for the storage this enzyme [22].

**Effect of temperature and thermal stability behaviour**

As far as the temperature dependence activity is concerned, many of the L-glutaminases reported to have both optimal and stable temperature of around 40°C to 50 °C. Based on the results, the purified L-glutaminase from *Bacillus* sp. B12 was active over a wide range temperature from 25°C to 50 °C (Fig.3). L-glutaminase kept about 75% if initial activity after incubation at 40 °C. A notable decrease in enzyme activity was observed at higher temperature above this value, at 60°C only 25% of L-glutaminase activity was still present. In this concern, Singh and Banik (2013) recorded that the purified L-glutaminase enzyme produced from *Bacillus cereus* MTCC 1305 showed greatest activity at 35°C.

The results of temperature effect on enzyme stability indicated that no significant enzyme activity was lost when it was pre-incubated at 55 °C for 60 min (Fig.3). In contrast with our result, Elshafei et al (2014) reported that the purified L-glutaminase from *Penicillium brevicompactum* NRC829 showed stability in range temperature from 50°C to 60°C [17].
Determination of the kinetic parameters $K_m$ and $V_{max}$

The kinetics parameters for L-glutaminase towards substrate was determined from the Lineweaver-Burk Plot. The affinity of the purified L-glutaminase to L-glutamine as substrate, with an apparent $K_m$ and $V_{max}$ values 0.4mmol/L and 0.133mmol/min, respectively (Fig.4). The low values of kinetic parameters ($K_m$ and $V_{max}$) indicated high affinity of the enzyme to glutamine meaning that the rate will approach $V_{max}$ more quickly [23]. The results were in agreement with that reported by Singh and Banik (2013), they revealed that Bacillus cereus MTCC 1305 L-glutaminase has the high affinity to L-glutamine and presents a small $K_m$ (0.129 mmol)[19]. Also, Elshafei et al [17] recorded the highest activity of Penicillum brevicompactum NRC829 L-glutaminase towards L-glutaminase, with $K_m$ value of 0.13 mmol.

Effect of metal ions and inhibitors on the activity and stability on the purified L-glutaminase

The impact of various inhibitors and activators on prepare L-glutaminase was evaluated by pre incubation of the enzyme with each compound for 60 min without substrate. Results in Table 2 demonstrated a variation in enzyme activity, the presence of Ca$^{2+}$, Na$^{+}$, Mn$^{2+}$, and Mg$^{2+}$ enhanced the enzyme activity (Table 2). Also, the enzyme retained more than 90% of the total activity in presence of K$^+$, Ni$^{2+}$, and Co$^{2+}$. However, a considerable loss of activity was observed with the Sn$^{2+}$, Zn$^{2+}$, Cu$^{2+}$, Fe$^{2+}$, Hg$^{2+}$, Fe$^{2+}$ and they inhibit more than 50% of the purified L-glutaminase activity (Table 2). Constantly with our results, Singh and Banik (2013), reported that Na$^+$, K$^+$ and phosphate ion activated the Bacillus cereus MTCC 1305 L-glutaminase; while, divalent cations Mn$^{2+}$, Mg$^{2+}$, Zn$^{2+}$, Pb$^{2+}$, Ca$^{2+}$, Co$^{2+}$, Hg$^{2+}$, Cd$^{2+}$, and Cu$^{2+}$ inhibited its activity[19]. The Mn$^{2+}$ (2mmol) acted as activator for L-asparaginase from Rhizomucor miehei [24].
Table 2. Effect of different metal cations salts & various compounds on L-glutaminase activity.

<table>
<thead>
<tr>
<th>Metal salts</th>
<th>Concentration (mM)</th>
<th>Relative activity activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NiSO4</td>
<td>10</td>
<td>98</td>
</tr>
<tr>
<td>NaCl</td>
<td>10</td>
<td>106</td>
</tr>
<tr>
<td>MgCl2</td>
<td>10</td>
<td>112</td>
</tr>
<tr>
<td>CaCl2</td>
<td>10</td>
<td>104</td>
</tr>
<tr>
<td>MnCl2</td>
<td>10</td>
<td>109</td>
</tr>
<tr>
<td>KCl2</td>
<td>10</td>
<td>96</td>
</tr>
<tr>
<td>FeSO4</td>
<td>10</td>
<td>48</td>
</tr>
<tr>
<td>CuSO4</td>
<td>10</td>
<td>34</td>
</tr>
<tr>
<td>ZnSO4</td>
<td>10</td>
<td>33</td>
</tr>
<tr>
<td>HgCl</td>
<td>10</td>
<td>48</td>
</tr>
<tr>
<td>SnCl2</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>CoCl2</td>
<td>10</td>
<td>95</td>
</tr>
<tr>
<td>Cysteine</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>Sodium Azide</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>EDTA</td>
<td>10</td>
<td>95</td>
</tr>
<tr>
<td>PMSF</td>
<td>10</td>
<td>98</td>
</tr>
<tr>
<td>2-Mercaptoethanol</td>
<td>10</td>
<td>25</td>
</tr>
</tbody>
</table>

Moreover, the presence of 2-Mercaptoethanol and Cystein (10mM) acted as inhibitors of L-glutaminase activity, the activity was reduced to around 70% of the total activity. On the other hand, PMSF and EDTA kept more than 90% of the enzyme activity. While, Sodium azid has no effect on the enzyme activity, which indicates that L-glutaminase might not be metalloenzyme (Table 2). Inhibition in the presence of thiol group blocking reagents indicates that the enzyme has thiol group(s) in the catalytic site of the enzyme.  

Acknowledgment: Authors are thankful to authorities of Diyala University, College of Science for providing the necessary facilities to complete the work successfully.

Source of Funding: The research Project was sponsored by the authors.

Ethical Clearance: No ethical approval was obtained because this study did not involve a prospective evaluation, did not involve laboratory animals.

Conflict of Interest: Nil

References


The Role of immunohistochemical Expression of monoclonal VE1 antibody in detecting the BRAF gene mutation in patients with colorectal carcinoma.

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Abstract

Purposes of study: to evaluate clinical parameters and histopathological features of Colorectal carcinoma (CRC) and its association with BRAF mutation as immunohistochemical markers in Babylon province.

Methods: This research is prospective study. The total patients are 42 Manual IHC staining procedure were done. Staining intensity were scored as: 0 (negative), 1 (weak diffuse staining in comparing to background staining), 2 (moderate diffuse staining), and 3 (strong diffuse staining). IHC scoring was regarding as positive when there was diffuse, homogenous and more than 80% of cytoplasmic staining area of cancer cells. Negative cases when there were absent staining or nuclear staining or weak isolated staining cells. Score 0 and 1 were consider negative and score 2 and 3 were positive. Results: Positive IHC staining of BRAF mutation was presenting more in older age group (65 ±15.23 years), male gender (60%) and left sided colon (60%) but there were insignificant association of these above parameters. Grade 2 and grade 3 of CRC was the highest frequency of positive BRAF cases (40%). Positive IHC staining for BRAF mutation expression is more frequency in T3, N1-2, and M0 stage, and stage III that shows 60%, 80%, 100%, 80% respectively. There were no significant association between BRAF IHC with TMN staging and grading systems. Conclusion: the current study found to be predominant in older age (> 65 years old), high grade (G2-3) and high stage (III). Parameters of high grade & stage associated with poor prognosis & high mortality outcome. BRAF gene IHC expression could be consider an independent bad prognostic factor for patients with CRC.

Key words: Colorectal carcinoma, BRAF gene, immunohistochemistry method.

Introduction

Colorectal carcinoma (CRC) is 3rd common malignancy in world(1) and one of leading cause of mortality in Western area of world(2). Tumor-node-metastasis (TNM) staging system is remain as prognostic parameter for this cancer(3).

The distribution of this tumor is equal regarding sex and mean affecting age is between 6th and 7th decades of life(4).

Both chronic inflammatory bowel diseases and Schistosoma mansoni infection are causes of CRC (3).

BRAF gene (v-Raf murine sarcoma viral oncogene homolog B) is an oncogene, undergo mutation, and found about 10%-15% of CRC(5,6). The common mutation in BRAF gene is V600E and account for about 80% (7), and found in colorectal adenocarcinoma (5%-15%), papillary type of thyroid cancer (45%), melanomas (40%-60%), serous type of ovarian cancer (35%), lung cancer (1%-3%) and other cancers(8).

CRC with BRAF oncogene mutation have recurrent association with poorly differentiation mucinous cancer and higher TNM staging system(9), and so, it can predict an essential role in treatment of CRC(10), and this BRAF mutation can distinguish between CRC of sporadic type
from hereditary non-polyposis type/Lynch syndrome\cite{11}.

Study aim was to evaluate clinical parameters and histopathological features of CRC and its association with BRAF as immunohistochemical markers in Babylon province.

**Method**

**Patients samples**

This research is prospective study. The patients with diagnosed CRC underwent clinical evaluation about age ,sex, metastasized CRC or not, if patients were taking chemotherapy or not, duration of CRC, and lastly, biopsy or total (or partial) colectomy.

These patients were selected from Al-Hilla Surgical Teaching Hospital, private Teeba Hospital, many private histopathological laboratory, and the specimens of CRC were partial or total colectomy, reported its large intestine site, and selected before chemotherapy. The paraffin embedded blocks of cancer were reviewed by two pathologists to ensure the diagnoses of CRC and were classified according to TNM staging system (8th edition, 2017)\cite{12} and WHO grading system (2000)\cite{13}.

The total patients (42) were collected between February/2017 to April/2019, 25 men and 17 women, and age ranged between 35-83 years old (median age 64).

**Immunohistochemistry (IHC)**

The BRAF V600E kit protocol was Bio SB, Inc., USA, BRAF VE1 antibody is Rabbit Monoclonal antibody (isotype IgG) that shows cellular cytoplasmic staining, Clone RM8, and Catalog No. BSB 2824.

IHC scoring of BRAF V600E was depend on percentage and intensity of staining. Staining intensity were scored as : 0 (negative), 1 (weak diffuse staining in comparing to background staining ), 2 (moderate diffuse staining), and 3 (strong diffuse staining) . Positive control ( melanoma cancer has BRAF mutation) and negative control ( usually making by removing primary antibodies ) were used with each run of IHC procedure.

IHC scoring was regarding as positive when there was diffuse, homogenous and more than 80% of cytoplasmic staining area of cancer cells. Negative cases when there were absent staining or nuclear staining or weak isolated staining cells. Score 0 and 1 were consider negative and score 2 and 3 were positive \cite{14,15}

**Statistical Analysis**

SPSS software (version 22) was statistical program. Categories data were indicated as frequency and percentage and were assessment by using Pearson’s chi. Continuous data represented as range , median, mean ± SD, and were measured by Independent T Test if the data is normal distributed . \( P \text{ value} < 0.05 \) was significant difference between two parameters.

**Results**

A total 42 patients with CRC ( adenocarcinoma type ), 25 men and 17 women, mean age±SD ( 63.36±12.21), and were assessment by using IHC staining of BRAF mutation. General clinicopathological features of present study are illustrated in Table 1.

\[
\begin{array}{|c|c|}
\hline
\text{Features} & \text{Total N (\%)} \\
\hline
\text{Age (years old)} & \\
35-54 & 5(11.9\%) \\
55-74 & 27(64.3\%) \\
75-83 & 10(23.8\%) \\
\text{Gender} & \\
Male & 25(59.5\%) \\
Female & 17(40.5\%) \\
\hline
\end{array}
\]
Cont.. TABLE 1: Clinicopathological basic characteristics in CRC patients (total n. =42).

<table>
<thead>
<tr>
<th>Tumor site</th>
<th>Left side colon</th>
<th>26 (61.9%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right side colon</td>
<td>10 (23.8%)</td>
</tr>
<tr>
<td></td>
<td>Rectum</td>
<td>6 (14.3%)</td>
</tr>
<tr>
<td>T stage</td>
<td>T1</td>
<td>8 (19%)</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>18 (42.9%)</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>16 (38.1%)</td>
</tr>
<tr>
<td></td>
<td>T4</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>N stage</td>
<td>N0</td>
<td>36 (85.7%)</td>
</tr>
<tr>
<td></td>
<td>N1-2</td>
<td>3 (7.1%)</td>
</tr>
<tr>
<td>M stage</td>
<td>M0</td>
<td>39 (92.9%)</td>
</tr>
<tr>
<td></td>
<td>M1</td>
<td>3 (7.1%)</td>
</tr>
<tr>
<td>Tumor stage</td>
<td>I</td>
<td>3 (7.1%)</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>3 (7.1%)</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>33 (78.6%)</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>3 (7.1%)</td>
</tr>
<tr>
<td>Tumor grade</td>
<td>G1</td>
<td>12 (28.6%)</td>
</tr>
<tr>
<td></td>
<td>G2</td>
<td>20 (47.6%)</td>
</tr>
<tr>
<td></td>
<td>G3</td>
<td>10 (23.8%)</td>
</tr>
<tr>
<td></td>
<td>G4</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

IHC staining procedure show 8 cases with BRAF staining and remaining 36 show no staining, 8 cases include strong intensity 2 cases, moderate intensity 3 cases, weak intensity 3 cases, and the staining percentage of these cases were between 80%-95%. So, positive expression of BRAF IHC in CRC were represented always moderate to strong staining (5 cases). While, negative cases were including weak and no staining cases.

Positive IHC staining of BRAF mutation was presenting more in older age group (65 ±15.23 years), male gender (60%) and left sided colon (60%) but there were insignificant association of these above parameters as in Table 2.
TABLE 2 : Association of clinical characteristics in CRC patients with IHC (total n. =42).

<table>
<thead>
<tr>
<th>Clinical data</th>
<th>BRAF IHC N(%)</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Age mean±SD</td>
<td>63±11.87</td>
<td>65±15.23</td>
</tr>
<tr>
<td></td>
<td>35-54</td>
<td>4(10.8%)</td>
</tr>
<tr>
<td>Age (years old)</td>
<td>24(64.9%)</td>
<td>3(60%)</td>
</tr>
<tr>
<td></td>
<td>75-83</td>
<td>9 (24.3%)</td>
</tr>
<tr>
<td>Gender</td>
<td>22(59.5%)</td>
<td>3(60%)</td>
</tr>
<tr>
<td></td>
<td>15(40.5%)</td>
<td>2(40%)</td>
</tr>
<tr>
<td>Tumor site</td>
<td>23(62.2%)</td>
<td>3(60%)</td>
</tr>
<tr>
<td>Left side colon</td>
<td>8(21.6%)</td>
<td>2(40%)</td>
</tr>
<tr>
<td>Right side colon</td>
<td>6 (16.2%)</td>
<td>0(0%)</td>
</tr>
</tbody>
</table>

^Pearson chi-Square
#Independent Samples Test

Grade 2 and grade 3 of CRC was the highest frequency of positive BRAF cases (40%). Positive IHC staining for BRAF mutation expression is more frequency in T3, N1-2, and M0 stage, and stage III that shows 60%, 80%, 100%, 80% respectively.

There were no significant association between BRAF IHC with TMN staging and grading systems as in Table 3.

TABLE 3 : Evaluation of histopathological features in CRC patients by IHC (total n. =42).

<table>
<thead>
<tr>
<th>Features</th>
<th>BRAF IHC N(%)</th>
<th>^P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Tumor grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>11(29.7%)</td>
<td>1(20%)</td>
</tr>
<tr>
<td>G2</td>
<td>18(48.6%)</td>
<td>2(40%)</td>
</tr>
<tr>
<td>G3</td>
<td>8(21.6%)</td>
<td>2(40%)</td>
</tr>
<tr>
<td>G4</td>
<td>0(0%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Tumor grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1-G2</td>
<td>29(78.4%)</td>
<td>3(60%)</td>
</tr>
<tr>
<td>G3-G4</td>
<td>8(21.6%)</td>
<td>2(40%)</td>
</tr>
<tr>
<td>T stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1-2</td>
<td>24(64.9%)</td>
<td>2(40%)</td>
</tr>
<tr>
<td>T3-4</td>
<td>13(35.1%)</td>
<td>3(60%)</td>
</tr>
<tr>
<td>T stage</td>
<td>T1</td>
<td>8(21.6%)</td>
</tr>
<tr>
<td>---------</td>
<td>----</td>
<td>----------</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>16(43.2%)</td>
</tr>
<tr>
<td></td>
<td>T3</td>
<td>13(35.1%)</td>
</tr>
<tr>
<td></td>
<td>T4</td>
<td>0(0%)</td>
</tr>
<tr>
<td>N stage</td>
<td>N0</td>
<td>5(13.5%)</td>
</tr>
<tr>
<td></td>
<td>N1-2</td>
<td>32(86.5%)</td>
</tr>
<tr>
<td>M stage</td>
<td>M0</td>
<td>34(91.9%)</td>
</tr>
<tr>
<td></td>
<td>M1</td>
<td>3(8.1%)</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>3(8.1%)</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>2(5.4%)</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>29(78.4%)</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>3(8.1%)</td>
</tr>
</tbody>
</table>

\^[Pearson chi-Square]

FIG. 1 : BRAF IHC staining in the CRC. (A) It is showing negative staining at10X. (B) It is diffuse cytoplasmic and weak staining (10X), (C) and It is diffuse and moderate staining (X10) , (D) and (E) It has diffuse and strong staining at 10X and 40x respectively.

**Discussion**

The main objective of this study is to evaluate BRAF gene mutation prevalence by utilizing monoclonal VE1 AB in colorectal carcinoma & compare the outcomes with different clinical & histopathological features. Many previous studies of BRAF gene showed good compatibility between IHC technique and genotype sequencing methods regarding many cancer types \(^{(16-23)}\), but Adackapara et al\(^{(24)}\) showed less compatibility (71% sensitivity and 74% specificity), this might be due to using manual IHC method with incubation overnight of IHC antibodies\(^{(25)}\).
BRAF mutation investigation is used as mandatory in practical laboratory\(^{26}\). Also, polymerase chain reaction technique required more time, need special structures, affected by method of preservation of formalin fixed paraffin tissue and tissue heterogeneity\(^{20}\), while, IHC is usually done in most pathological laboratories, less costly, and less complex procedure in compare to genotype procedure, lastly, genetic procedure for BRAF mutation can be regarded as critical method only when there are an equivocal IHC BRAF cases to confirm or exclude their positivity\(^{27}\).

Also, Sinicrope et al\(^{(15)}\) used BRAF scoring as negative when there are nuclear staining or weak separated cancer cells with cytoplasmic staining, and positive scoring when there are 100% homogenous cytoplasmic staining area of cancer cells in 75% of patients or more than 70% of stained cells from total cases as scoring standard as in our research.

In the present study, there were 5 positive cases for VE1 (11.9%) most of them in older age and commonly in age group 55-74 years, male gender, and left side of colon (tumor site), TNM stage (T3, N1-2, M0), tumor grade (G2-3), however; no significant association between all these parameters and BRAF IHC results were observed.

Christian et al \(^{28}\) (cohort 1) demonstrated that positive IHC BRAF cases (13.5%) were more in older age, female gender, left sided colon cancer, TNM stage (T3-T4, N0, and M0), and tumor grade (G1-2), All these factors (gender, tumor site, TNM tumor stage, and tumor grade) showed insignificant association with BRAF IHC.

Fiona et al \(^{29}\) demonstrate that positive BRAF IHC (13.2%) expressed higher in female, right colon cancer, tumor grade 3, and tumor stage II-III.

Several studies of BRAF IHC showed result positivity more in older age, female sex, righted sided CRC, and more advanced tumor stage\(^{30-33}\).

In conclusion, the current study is the first Iraqi study provide new information about the BRAF V600 gene mutation prevalence among Iraqi patients with CRC utilizing IHC technique, it found to be predominant in older age (> 65 years old), high grade (G2-3) and high stage (III). Parameters of high grade & stage associated with poor prognosis & high mortality outcome, then BRAF gene IHC expression could be consider an independent bad prognostic factor for those patients with CRC.

**Acknowledgement:** The authors dedicate thanks and gratitude to the physicians and health staffs of Al-Hilla Surgical Teaching Hospital for supporting our research.

**Ethical Clearance:** this research was done and applied according to standards national research committee of our university and country.

**Source of Funding:** this research was funded by the three authors above.

**Conflict of Interest:** The authors announce that they have no conflict of interest.

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Evaluation the Effects of *Zingiber officinale* L. as a feed Additive on Growth and Some Serum Biochemical Profiles of *Cyprinus carpio* L

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Abstract

The research was performed to examine the efficacy of *Zingiber officinale* L. as feed additives on growth performance and biochemical parameters in common carp *Cyprinus carpio* L. Used (80) fish and divided into 4 groups 10 fish per aquaria, two replicates were maintained for each treatment. Ginger powder mixed directly with basal diet of fish. It was incorporated in the four diets treatments. The control group diet had no ginger additive (C), while the three supplemented diets contained ginger at 1% (T1), 1.5 (T2) and 2 % (T3). After 35 days’ growth was calculated as weight gain, daily weight gain, relative growth ratio, feed conversion ratio, feed efficiency percent and protein efficiency ratio with evaluate total proteins, albumin, globulin, cholesterol and triglycerides. The results of present study were weight gain of T2 and T3 groups showed significant difference comparison with the C. Daily weight gain showed significant difference between C, T2 and T3. Relative growth rate there were no significant difference between C and T1 but there was significant difference between T2 and T3. Feed conversion ratio showed no significant difference between control group and T1. Feed efficiency ratio there were no significant difference between T1, T2 and C. Protein efficiency ratio results showed that decrease in T3 and T2 compared to the C. The results of total protein, albumin, and triglycerides did not show any significant difference between treatments compared with the C. Globulin showed increase in T3 compared to C. While the cholesterol showed significant difference in T3 compared with C, T1 and T2. The results of present study propose that ginger feed additives at the concentration 2% for 35 days can be improvement growth and some serum biochemical parameters.

**Keywords:** *Zingiber officinale* L., growth performance, biochemical parameters, *Cyprinus carpio* L

Introduction

Feed additives used to improve the growth and health of aquaculture species. Extensive use of antibiotics and biocides in aquaculture lead to antibiotic-resistant and generation of toxicants which may cause risks to the environment \(^[1]\). Consequently, The need of safe and effective alternatives to antibiotics is required, increasing attention to the use of natural alternative feed additives as supplementing ginger in fish diet \(^[2]\).

*Zingiber officinale* L. has been used as a spice for over 2000 years \(^[3]\). It is also called “The Great Medicament” in Ayurvedic medicines \(^[4]\) and is generally considered as a safe herbal medicine \(^[5]\). Fresh ginger contains 80.9% moisture, 2.3% protein, 0.9% fat, 1.2% minerals, 2.4% fiber and 12.3% carbohydrates.

Ginger contain natural organic materials for control disease in aquaculture by increasing the non-specific and specific immune mechanisms \(^[6]\). Supplementing diets ginger was reported to enhance the growth of tilapia (Oreochromis mossambicus) \(^[7,8]\). The digestive enzyme activity significantly increased with ginger enrichment \(^[9]\). \(^[10]\) reported *Zingiber officinale* in diet of koi carps increase its production and enhance growth. Ginger has been reported to possess a broad-spectrum of prophylactic and therapeutic activities \(^[11,12,13]\). Serum total proteins are indicator of the biochemical nutritional and health status of the fish \(^[14]\). The use of ginger powder as supplemented diet can cause the increase of total protein in Lates calcarifer \(^[15]\). Studies have reported increase in serum albumin and globulin with work relating to fish fed with ginger diet \(^[10]\). The aim
of the study was to assessment the effects of ginger as a feed additive in the diets of common carp Cyprinus carpio L. on growth and serum biochemical parameters.

**Materials and Method**

A total of (80) healthy fish of Cyprinus carpio L. average weight (75 ± 2 g) were obtained from a carp farm at Aljadera - Diyala (Iraq) were used in the experimental study. They were stocked in a two path through (150 x 80 x 50 cm) then the fish were selected randomly and dispersed in to the 8 glass aquaria 10 fish per aquarium for each treatment. The ginger was mixed directly with basal diet of fish. It was incorporated in the four diets treatments. The control diet had no ginger additive, while the three supplemented diets contained ginger at 1% (T1), 1.5 (T2) and 2 % (T3). All fish were fed 3% of their body weight two times daily for 35 days. Growth performance parameters calculated via the following equation: Body weight gain (WG) = final fish weight (g) – initial fish weight (g) [16]. Daily body weight gain (DG) = Gain (g) / time (day) [17]. Relative growth ratio (RGR%): final fish weight (g) – initial fish weight (g) / time (day) X 100 [18]. Feed conversion ratio (FCR) = feed intake (g) / Weight gain (g) [17]. Feed efficiency percent (FER %) = Weight gain (g) X 100 / feed intake [19]. Protein efficiency ratio (PER) = Weight gain (g) / Protein intake (g) [19]. At the end of the experiment, blood was drawn from caudal vessels using a non-heparinized syringe and collected in plastic gel tube. The evaluate of serum total proteins was carried by colorimetric method described by [20]. While estimation of albumin was carried according to [21]. Globulin content was calculated by subtracting albumin value from serum total protein value. Cholesterol and triglycerides were carried by using laboratory diagnostic kits.

**Statistical Analysis**

The statistical analysis system [22] program was used to show the effect of different treatments in study parameters. Least significant difference –LSD test and ANOVA were used to show the significant differences and compare between means in this study.

**Result**

The mean body weight gain (BW), Daily body weight gain (DG), Relative growth ratio (RGR). Food conversion rate (FCR), Food conversion efficiency (FCE) and Protein Efficiency ratio % (PER) were reported in Table (1). The body Weight gain (g/fish) of T2 and T3 groups showed significant difference at (P≥0.05) (4.81±1.121, 7.99±1.00) respectively with the control group (0.72±0.15) and T1(1.42±0.23) on the other hand there were statistically significant difference between T2 and T3. Daily weight gain showed significant difference at (P≥0.05) between T2, T3, C and T1 were (0.24±0.033, 0.66±0.070) and (0.03±0.005, 0.07±0.019) respectively. Relative growth% there were no statistically significant difference at (P≥0.05) between control and T1 (10.03±0.30, 11.86±0.52) respectively but there was statistically significant difference at (P≥0.05) between T2 and T3 (14±0.61, 17.39±0.86) respectively. Feed conversion Ratio (FCR) showed no significant difference at (P≥0.05) between control group and T1 (18.55±0.54, 17.2±1.03) respectively but there were significant difference between T3 and C, T1, T2 with low FCR in T2 and T3 (15.57±0.93 and 11.57±0.63) respectively. Feed efficiency ratio % there were no statistically significant difference at (P≥0.05) between control group, T1 and T2 was (1.85±0.30, 1.52±0.21, 3.08±0.47) respectively while T3 (6.16±1.05) showed the greatest value and were statistically significant difference at (P≥0.05) with C, T1 and T2. Protein efficiency ratio % results showed that decrease in T3 and T2 values in fish fed ginger diet (P<0.05) compared to the control and T1 (1.12±0.007, 2.25±0.08 and (5.17±0.12, 5.37±0.31) respectively.

The total protein, albumin, and triglycerides did not show any significant difference between treatments compared with the control group (P≥0.05). Globulin showed significant increase in T3 (0.47±0.04) compare to C (0.28±0.03). While the cholesterol showed significantly reduces in T3 (71.16±3.70) compare with C1, T1, T2 (83.66±3.79, 82 ±2.63, 73.16±5.28) respectively (Table 2).
Table (1) Effect of ginger on growth performance (weigh gain, daily weigh gain, relative growth %, feed conversion %, feed efficiency % and protein efficiency %) of C. carpio fed different concentrations of ginger for 35 days. Similar letters represent non-significant difference at P ≥ 0.05. Value represent Mean ±SE.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body Weigh Gain</th>
<th>Daily Weigh Gain</th>
<th>Relative Grouth %</th>
<th>Feed Conversion %</th>
<th>Feed Efficiency %</th>
<th>Protein Efficiency %</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.72±0.15</td>
<td>0.03±0.005</td>
<td>10.03±0.30</td>
<td>18.55±0.54</td>
<td>1.85±0.30</td>
<td>5.17±0.12</td>
</tr>
<tr>
<td>T1</td>
<td>1.42±0.23</td>
<td>0.07±0.019</td>
<td>11.86±0.52</td>
<td>17.2±1.03</td>
<td>1.52±0.21</td>
<td>5.37±0.31</td>
</tr>
<tr>
<td>T2</td>
<td>4.81±1.121</td>
<td>0.24±0.033</td>
<td>14±0.61</td>
<td>15.57±0.93</td>
<td>3.08±0.47</td>
<td>2.25±0.08</td>
</tr>
<tr>
<td>T3</td>
<td>7.99±1</td>
<td>0.66±0.070</td>
<td>17.39±0.86</td>
<td>11.57±0.63</td>
<td>6.16±1.05</td>
<td>1.12±0.007</td>
</tr>
<tr>
<td>LCD 0.05</td>
<td>2.611</td>
<td>0.133</td>
<td>1.993</td>
<td>2.511</td>
<td>1.972</td>
<td>0.574</td>
</tr>
</tbody>
</table>

Table 2: Blood serum biochemical parameters of C. carpio after 35 days feeding different doses Ginger (1%, 1.5%, 2%) compared with the control group. Similar letters represent non-significant difference at P ≥ 0.05. Value represent Mean ±SE.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total protein g/dl</th>
<th>Albumin g/dl</th>
<th>Globulin g/dl</th>
<th>Cholesterol mg/dl</th>
<th>Triglyceride mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>1.83±0.07 A</td>
<td>1.49±0.04 A</td>
<td>0.28±0.03 B</td>
<td>83.66±3.79 A</td>
<td>116.16±4.02 A</td>
</tr>
<tr>
<td>T1</td>
<td>1.98±0.19 A</td>
<td>1.44±0.04 A</td>
<td>0.38±0.01 AB</td>
<td>82.±2.63 AB</td>
<td>116.16±11.12 A</td>
</tr>
<tr>
<td>T2</td>
<td>2.05±0.09 A</td>
<td>1.45±0.05 A</td>
<td>0.41±0.04 AB</td>
<td>73.16±5.28 AB</td>
<td>116±6.21 A</td>
</tr>
<tr>
<td>T3</td>
<td>2.08±0.11 A</td>
<td>1.40±0.02 A</td>
<td>0.47±0.04 A</td>
<td>71.16±3.70 B</td>
<td>96.66±4.73 A</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>0.377</td>
<td>0.131</td>
<td>0.186</td>
<td>11.704</td>
<td>20.920</td>
</tr>
</tbody>
</table>
Discussion

Highest supplementation of ginger in T3(2%) achieved the best significant final average body weight followed by fish groups fed on diet contained (1.5% - 1% mg /kg) ginger respectively, while the lowest values were in control group similar results observed that beluga fed diet with ginger powder significantly increased growth performance [23]. These results clearly showed that the ginger stimulated fish growth may be respond to ginger supplementation in a dose dependent manner. These results are also in accordance with [24] who suggesting highest supplementation of ginger was most favorable for the growth and survival. Also, [25] administration of ginger in grouper enhanced innate immune defenses. The enhanced growth response indicated by gainer supplementation stimulates protein synthesis by enzymatic system [27,26] and agreement with [28] who reported that goldfish fed ginger supplemented diets, enhanced growth. The result of the FCR low in T2 and T3 (15.57±0.93 and 11.57±0.63) respectively it is in contrast with that of [29] who reported that there was a significant difference in Feed conversion ratio in shrimp fed ginger supplemented. These values are also observed in other teleosts [30] and tilapia [31]. Previous study significantly increased albumin and globulin are vital elements for maintaining a healthy immune system [32]. This is in agreement with finding of [33, 34] an increase in serum protein levels as an indication of strong innate immune response in fish, likewise [35] showed higher levels of albumin and globulin. Ginger had no significant difference on triglyceride compared with the control (Table 2). In the present study, total protein and albumin was not change after feeding with different doses of ginger at the end of 35 days and globulin had significant difference in fish fed diet containing ginger when compared with the control.

Conclusion

The research concluded that ginger supplemented diet at the concentration 2% for 35 days improved growth and higher levels of globulin with reduce in both cholesterol and triglyceride in blood serum analyzer. Further research is needed to clarify the action mechanism of ginger and feeding period in common carp.

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Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Disclosure of potential conflicts of interest and current submission:

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other peer-reviewed media.

Informed consent: “Informed consent was obtained from all individual participants included in the study.”

Source of Funding: Self-funding.

Ethical Clearance: Not Required.

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The Infestation Study of *Oestrus ovis* L. 1761 in Sheep of Al-Amara Region, Maysan Province, South of Iraq

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Abstract

362 heads of sheep obtained (in multiple visits) from the central slaughterhouse and the sheep abattoir at Al-Amara region (Maysan, South of Iraq) between November 2016 and March 2017 were examined for the detection of *Oestrus ovis* larvae for the first time in this region. Sheep heads were examined by dissecting them longitudinally, the sheep were of local breed. Of the 362 heads, 296 (81.8%) were infested with *O. ovis* larvae. *O. ovis* larvae were observed recorded in both sexes and all age groups in different temperature ranges (low, medium and high) during the research period. A total of 985 larvae were collected. The overall infestation intensity (IN) was 5.443% L/H with a range of 2-8 larvae per infested head of sheep (L/Hs). The infestation of larval in the infested sheep was 54.14% in low-temperature weather, while 100% in relatively high-temperature weather. March showed the highest infection intensity, while January showed the lowest infection intensity. The novelty of the current study is to conduct the research in an area that totally different from what has been studied before in Iraq in regards to the geographical location and the climate conditions.

Keywords: *Oestrus ovis*, Oestrosis, infestation, sheep.

Introduction

*Oestrus ovis* (Linne 1761, Diptera: Oestridae) the sheep nasal bot fly, is a well-known parasite of the cavities and adjoining sinuses in sheep and goats[1]. *O. ovis* has a worldwide distribution and is connected with a severe parasitosis of small ruminants, with emphasis particularly in the Mediterranean regions and Middle East. The growth of larvae in the nasal sinus cavities may guide to serious clinical signs that together with the irritation caused by the larvae of bot fly may result into severe economic losses[1]. Moreover, *O. ovis* infestation may be considered a zoonotic parasite[2] as it may cause ophthalmyiasis in human as reported in many parts of the world including Libya[3] and other Middle Eastern countries such as Jordan, southern Iran, northern Iraq, Saudi Arabia and Libya[4,5,6,7,8]. The pathogenicity of *O. ovis* larvae has frequently been clarified to cause mechanical and traumatic harm to the nasal mucus by the oral hooks and cuticular spines of the larvae that leads to inflammation and induces secondary infection[1].

Oestrosis causes nasal discharge that affects breathing especially in Iraq due to hot and dry weather and it may migrate from the nasal cavities and sinuses into brain causing false gid[9]. The sheep bot fly larva of *Oestrus ovis* is a mammalian parasite of the skin, nose, ears, and eyes[2]. When the larvae infest and feed on the external parts of the eye, the case is called ophthalmomyiasis. A number of cases of ophthalmitis or nasal/pharyngeal myiasis in humans caused by the oestrosis larvae have been reported with high infestation from numerous regions all over the world[3].

Several studies on *O. ovis* have been reported from the northern cities in Iraq[10] and in some Arabic countries[4,11,12]. In addition to some neighbor countries such as Iran[13], Turkey[14,15]. The infestation of *O. ovis* in sheep in Europe varies from 21.9% to 93.7%[16], 28.4% in France[1], and 45% in Spain[17].

To the best of our knowledge, there is no information on the intensity and incidence of *O. ovis* infestation available to the sheep in Southern Iraq despite the popularity of sheep breeding, therefore, this study was undertaken to investigate the infestation and intensity of infestation by *O. ovis* in sheep in Maysan Province/ southern of Iraq, in order to plan a cooperation with the
Ministry of Agriculture to get rid of this parasite.

**Materials and Method**

**Animals and study area:** 362 heads of sheep were slaughtered and examined for *O. ovis* infection from November 2016 to March 2017 at the slaughterhouse and abattoirs located in the Maysan province. The area lies north-east of Basra city and south of Baghdad (the capital city of Iraq) and located on longitude 31.8496° N and attitude 47.1460° E.

**Examination procedure:** Individual heads of the slaughtered animals were separated from the carcasses, all information such as the date of sampling, the cod number of the head, the result of the examination and the number of larvae in each head were recorded. The heads were cut along their longitudinal and sagittal axes using dissecting equipment with assisting of the butchers. After slaughter, the heads were put separately into nylon bags and transported to the laboratory for examination. All nasal cavity (the nasal passage, septum, middle meatus, and conchae) and paranasal sinus (frontal and maxillary) of each head were carefully examined. The larvae of insect *O. ovis* was isolated and collected into vials and transferred to the laboratory. After collection of the larvae, 1% hydrogen peroxide (H$_2$O$_2$) was injected through the breathing hole of the larvae and was preserved in 70% ethanol as described by[18]. The larvae were counted and recognized according to[19]. The intensity of the infestation (IN) was calculated based on number of larvae/ number of infested animal heads (L/Hs).

**Statistical analysis:** The collected data were analyzed statistically using the chi-square test, standard deviation, percentage, and F-test using the Statistical Package for Social Sciences (SPSS). All data was rested for normal distribution and submitted to one-way ANOVA.

**Results and Discussion**

The *Oestrus ovis* fly had been known to be epidemic in Iraq for long time ago; however, only a limited data on its infestation is available and have been reported from the Northern Iraqi Provinces[10,20,21,24]. Hence, this study was conducted for the first time to calculate the incidence of this parasite in Southern of Iraq which is totally dissimilar to the above studies in regards to the geographical location and the climate conditions[22]. The activity of this parasite is strongly influenced by the climatic factors[23].

The investigation for *O. ovis* larvae had been made during the months of November, December, January, February, and March (2016/2017) (Table 1). Out of the examined 362 sheep heads, around 81.8% were found to be infested with *O. ovis* larvae (Figure 1). This prevalence of infestation rate of sheep was higher than those recorded in Northern of Iraq[10,20,21,24,25]. These recorded rates are also higher than to what have been conducted in other regions of the world[4,12,13,14,15,26,27,28,29,30,31,32]. This might be due to differences in geographical location, livestock breeding, animal race, animal age, host resistance, size of sheep flock, veterinary care and previous treatment with drugs, and environmental condition[4,21,23].

The current study showed the monthly prevalence of infestation ranged from 58.1% in January to 100.0% in March (Table 1) the total monthly results revealed high significant differences in the *O. ovis* infestation among the months of study (F= 11.773; p< 0.001). Temperature degrees[22] in the studied area showed in (Table 2).

During summer, the activity of the adult *O. ovis* fly increases[32], while during winter, the larvae suffer diapause periods, and decrease in their growth rate[1]. These findings coincided with previous reports[15,26]. In addition, the recorded larvae numbers actually return to an older formation (few months ago) due to the life cycle of such parasites. It was confirmed that 20 °C was the minimum temperature for the parasite activity, while most favorable temperature appears to range between 26 °C and 28 °C[23]. Consequently, the temperature were optimal for the parasite during February and March.

The morphological characteristics of the larval stage were displayed in Figure 2 and the most frequent larvae sizes were displayed in Figure 3. The present study showed that all larval instars were recovered (L1,L2and L3 instars which were detected clearly, but the L2 and L3 instars were the most recovered larvae (Table 3 and Figure 4). Many prior reports had provided similar observation in Iraq[21,24], and other countries[4,14,15,28]. It was observed that sheep, over two years old, has acquired immunity against *O. ovis* larvae as a result of repeated exposure to infestation while lambs remain more susceptible to infestation[31]. The small size of L1 larvae made them disappear in small places such as the ethmoid bone, during the examination period[28]. A proportion of *O. ovis* L1 larvae may develop inside the nasal cavities.
in the cold months, this explains the disappearance of the adult stage at this time with probability that expelled larvae either die or winters as pupal diapauses[32]. Therefore, the *O. ovis* adjusted its life cycle according to the surrounding local climate[15,28].

The mean of infestation intensity of infested sheep (IN) was 5.433 larvae per infested sheep head (L/SH) (Table 3). These results might return to the same factors that affected the distribution of this fly which explained by[4,21,23].

### Table 1. The monthly infestation of *Oestrus ovis* in sheep.

<table>
<thead>
<tr>
<th>Months</th>
<th>Total of examined animals</th>
<th>Number of infested animals</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>November</td>
<td>37</td>
<td>29</td>
<td>78.4</td>
</tr>
<tr>
<td>December</td>
<td>85</td>
<td>60</td>
<td>70.6</td>
</tr>
<tr>
<td>January</td>
<td>43</td>
<td>25</td>
<td>58.1</td>
</tr>
<tr>
<td>February</td>
<td>132</td>
<td>117</td>
<td>88.6</td>
</tr>
<tr>
<td>March</td>
<td>65</td>
<td>65</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>362</td>
<td>296</td>
<td>81.8</td>
</tr>
</tbody>
</table>

### Table 2.: The infestation of *Oestrus ovis* larvae in sheep with regards to Temperature range.

<table>
<thead>
<tr>
<th>Temperature range Cº</th>
<th>Number of Examined Sheep</th>
<th>Number of Infested Sheep</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-19</td>
<td>165</td>
<td>114</td>
<td>69.09</td>
</tr>
<tr>
<td>20-39</td>
<td>197</td>
<td>182</td>
<td>92.3</td>
</tr>
<tr>
<td>Total</td>
<td>362</td>
<td>296</td>
<td>81.8</td>
</tr>
</tbody>
</table>

### Table 3. The frequency of larval (IR) burden of *O. ovis* among the infested heads of sheep distributed in months of the study

<table>
<thead>
<tr>
<th>month</th>
<th>Number of larvae in infested heads (L/H)</th>
<th>Total</th>
<th>Uncounted heads</th>
<th>Non-infested heads</th>
<th>Total heads</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Nov.</td>
<td>7</td>
<td>14</td>
<td>3</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>8.0</td>
<td>12</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>Dec.</td>
<td>1</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.0</td>
<td>8</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Jan.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>25</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Cont.. Table 3. The frequency of larval (IR) burden of *O. ovis* among the infested heads of sheep distributed in months of the study

<table>
<thead>
<tr>
<th>Feb.</th>
<th>No. of heads</th>
<th>0</th>
<th>90</th>
<th>12</th>
<th>28</th>
<th>17</th>
<th>33</th>
<th>0</th>
<th>90</th>
<th>5.789</th>
<th>27</th>
<th>15</th>
<th>132</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Σ of larvae</td>
<td>0</td>
<td>51.1</td>
<td>48</td>
<td>140</td>
<td>102</td>
<td>231</td>
<td>0</td>
<td>521</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>March</td>
<td>No. of heads</td>
<td>0</td>
<td>65</td>
<td>15</td>
<td>15</td>
<td>18</td>
<td>17</td>
<td>0</td>
<td>65</td>
<td>5.569</td>
<td>0</td>
<td>0</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>Σ of larvae</td>
<td>0</td>
<td>36.9</td>
<td>60</td>
<td>75</td>
<td>108</td>
<td>119</td>
<td>362</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>No. of heads</td>
<td>8</td>
<td>176</td>
<td>32</td>
<td>48</td>
<td>35</td>
<td>50</td>
<td>1</td>
<td>176</td>
<td>5.596</td>
<td>120</td>
<td>66</td>
<td>362</td>
</tr>
<tr>
<td></td>
<td>Σ of larvae</td>
<td>16</td>
<td>100</td>
<td>128</td>
<td>240</td>
<td>210</td>
<td>350</td>
<td>8</td>
<td>985</td>
<td>-</td>
<td>0</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

X² = (229.868; df = 28); p < 0.0001

Figure 1: A sagittal section sheep head showing different larval stages of the nasal pot (the blue arrow: L2, the black arrow: L3).

Figure 2: The three larval instars of *O. ovis* in the nose of sheep. A-B: Second larval instar (L2), C: initial stages of third larval instar (L3), D-F: more advanced stages of L3.

Figure 3: The most frequent larvae sizes found in the current study.
Conclusion

The infestation of oestrosis in the sheep at Southern Iraq has been determined for the first time and *O. ovis* larvae were detected in 81.8% of the sheep examined. Infection by *O. ovis* is considered to be an important problem in sheep in Maysan province and is expected to be of importance for animal production and welfare. In order to estimate the economic significance of oestrosis, the efficiency of control measures and determines the precise infestation of *O. ovis* infestation in this province, further examinations are required including more animals and determining more risk factors. Finally, in order to lower the infestation rate of oestrosis it would be essential to use an effective and persistent drug against *O. ovis* in order to achieve eradication of the disease. In conclusion, it is possible to state that more than a few annual generations of adult flies may arise in the region. Novel studies will be essential to assess the impact of oestrosis in sheep production, as well the most excellent strategies of prophylaxis.

Acknowledgment: we would like to thank the staff at the main slaughterhouse at Maysan Province for helping us collection the sheep heads.

Financial Support and Sponsorship: Nil.

Conflict of Interest: There are no conflicts of interest.

Ethical Clearance: Permission to conduct this study was issued by the Health institutional and the main slaughterhouse authorities. Heads collection was carried out under veterinary public health technician supervision.

References


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Development and Preparation of ciprofloxacin Drug Derivatives for Treatment of Microbial Contamination in Hospitals and Environment

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Abstract

This practical studying involved preparation and development of new medical derivatives of ciprofloxacin drug via series of chemical reactions to preparation new derivatives to resist types of bacteria in hospitals and environment. Various chemical routes have been used to prepare ciprofloxacin derivatives, then all these derivatives reconnoitered with many spectral chemical techniques for instance (FT.IR, H.NMR, C.NMR) – spectrophotometric, then studying of their effects on bacterial pollution.

Keywords: Pollution, Bacteria, Ciprofloxacin, Environment.

Introduction

Ciprofloxacin is cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-quinoline-3-carboxylic acid as chemical name[1] while the brand names are (Cipro, Ciprobay, Ciproxan, Ciprinol), its formula(C₁₇H₁₈FN₃O₃), it is solid(Zhanel et al 2006., Heidelbagh and Holmster. 2013) [1,2] powder has faint to light yellow colour with melting [3,4] point (255-257 °C)[5].

![Ciprofloxacin](Fig.1:Ciprofloxacin Drug)

Ciprofloxacin medication is a broad-variety fluoroquinolone antibiotic[5, 6] secondhand in the dealing of a wide series(Oliphant C and Green. 2002) of slight to reasonable gram-(positive & negative) toxicities., which is used to indulgence or inhibit(Stevens et al 2005., Osmon et al 2013) certain infections affected through bacteria like gonorrhea; pneumonia[8-11] and toxicities(Vardakas K et al 2008., Donaldson P et al 1994) or infection of the skin, bone, joint, stomach[12-16] region, and( Karageorgopoulos et al 2008., Chow et al 2012., Stephenson et al 2013)

Prostate ciprofloxacin treatment is likewise used to delight or avoid and inhibit a serious infection that may be extent on tenacity[17-22] (Corrao G et al 2006., Liu X et al 2017) as portion of a bioterror round) and mouthful[23-27] (Bolhuis et al 2001., Pommier et al 2010., Goossens et al 2007) anthrax[28].

Experimental Part

Ciprofloxacin derivatives were inspected and identified thru: FT-IR spectra (FT-IR 8300 Shimadzu) in the range (400-4000) cm⁻¹ as KBr discs., H.NMR–Spectra and C.NMR in DMSO–solvent, in addition to resistance of ciprofloxacin derivatives against microbial pollution in hospitals and environment to improve their effect.
Preparation of Ciprofloxacin drug derivative

Preparation and development of Ciprofloxacin drug derivative\{1\}:

Ciprofloxacin drug developed by this paper thru preparation of its derivative\{1\} via three components reaction by following many chemical reactions like condensation reactions according to procedures\[29-33\] (Nagham 2016 .,Nagham et al 2015)to vintage precipitation, filtered ,dried and re crystallized to provide ciprofloxacin derivative\{1\}.

Preparation and development of Ciprofloxacin drug derivative\{2\}:

Ciprofloxacin drug developed to new drug by this study thru synthesis of its derivative\{2\} via azotation reaction,amination reaction ,then formazanation reaction by following many steps according\[29,30\] to procedures(Nagham et al 2015.,Intisar and Nagham 2018)to format precipitation, filtered ,dried and re crystallized to provide ciprofloxacin derivative\{2\}.

Preparation and development of Ciprofloxacin drug derivative\{3\}:

Ciprofloxacin drug developed via this work thru preparation of its derivative\{3\} via three components reaction, cyclization reaction, azotation ,amination reaction ,then formazanation step by following\[29,30\] procedures(Nagham et al 2015.,Intisar and Nagham 2018)to yield precipitation, filtered ,dried and re crystallized to provide ciprofloxacin derivative\{3\}.

Preparation and development of Ciprofloxacin drug derivative\{4\}:

Ciprofloxacin drug developed to derivative\{4\} via imination with formazanation reaction ,then three components reaction with triazole derivative according\[29,30\] to procedures(Nagham et al 2015.,Intisar and Nagham 2018)to give precipitation\[35\],filtered ,dried and re crystallized to produce ciprofloxacin derivative\{4\}.

Preparation and development of Ciprofloxacin drug derivative\{5\}:

Ciprofloxacin drug developed by this paper thru preparation of its derivative\{5\} via three components reaction, esterification, cyclization, imination reaction by following many chemical reactions like condensation reactions according\[34-36\] to procedures (Nagham 2016 .,Nagham et al 2015)to vintage precipitation, filtered ,dried and re crystallized to produce ciprofloxacin derivative\{5\}.

Results and Discussion

The developed ciprofloxacin derivatives studied and identified with multiplicity spectral techniques represented by (FT.IR ,H.NMR ,C.NMR) spectra with microbial tests and bacterial resistance studying:

Identification via Spectral Techniques :

FT.IR-Spectra of Ciprofloxacin Derivatives:

**1H.NMR-Spectra of Ciprofloxacin Derivatives:**

Our spectra provided new signals point to prepared drug derivatives and formatted functional groups(Nagham et al 2015.,Intisar and Nagham 2018) in this work, the spectra of all derivatives appeared signal at (2.5) for solvent (DMSO), novel derivative{1} appeared many signals at Illegal signal (COOH) Protons of carboxyl group:12.10 .,(NH)proton of amine:5.01 ..Protons of aromatic ring:(6.68-7.24)., (N-CH2-N) protons:3.64 .,(N-CH2-CH2-N) protons: (2.95-3.46) ..(CH=CH) proton of chalcone: (5.49).,(CH=CH-CH) protons of three membered ring:(0.83-1.46), but derivative{2} appeared many signals at Illegal signal (COOH) Protons of carboxyl group:11.59 .,(NH)proton of amine:5.12 ..Protons of aromatic ring: (6.93-7.78) ..,(N-CH2-CH2-N) protons: (2.61-3.54) ..(CH=C-CO) proton of chalcone: (5.56).,(CH2=CH2-CH) protons of three membered ring:(0.64-1.52), while derivative{3} appeared many signals at Illegal signal (COOH) Protons of carboxyl group:5.07 ..Protons of aromatic ring:(6.88-7.58), (N-CH2-CH2-N) protons: (2.75-3.41) ..(CH=C-CO) proton of chalcone: (5.67).,(CH2=CH2-CH) protons of three membered ring:(0.73-1.89).,(N-CH2-N) protons:3. 82 ..derivative{4} appeared many signals at Illegal signal (NH)proton of amine:5.12 ..Protons of aromatic ring: (6.93-7.78) ..,(N-CH2-CH2-N) protons: (2.61-3.54) ..(CH=C-CO) proton of chalcone: (5.56).,(CH2=CH2-CH) protons of three membered ring:(0.64-1.52), while derivative{3} appeared many signals at Illegal signal (COOH) Protons of carboxyl group:5.07 ..Protons of aromatic ring:(6.88-7.58), (N-CH2-CH2-N) protons: (2.75-3.41) ..(CH=C-CO) proton of chalcone: (5.67).,(CH2=CH2-CH) protons of three membered ring:(0.73-1.89).,(N-CH2-N) protons:3. 82 ..derivative{4} appeared many signals at Illegal signal (NH)proton of amine:5.12 ..Protons of aromatic ring: (6.93-7.78) ..,(N-CH2-CH2-N) protons: (2.61-3.54) ..(CH=C-CO) proton of chalcone: (5.56).,(CH2=CH2-CH) protons of three membered ring:(0.64-1.52), while derivative{3} appeared many signals at Illegal signal (COOH) Protons of carboxyl group:5.07 ..Protons of aromatic ring:(6.88-7.58), (N-CH2-CH2-N) protons: (2.75-3.41) ..(CH=C-CO) proton of chalcone: (5.67).,(CH2=CH2-CH) protons of three membered ring:(0.73-1.89).,(N-CH2-N) protons:3.90.

**The 13C.NMR spectral of Derivatives:**

Our spectra provided new signals point to prepared drug derivatives and formatted functional groups in this work., at the spectra of all derivatives appeared signal at (40.0) for solvent (DMSO), novel derivative{1} appeared many signals at (172.5) for (C ,carboxyl group COOH), (114.9-148.4) for (C ,Aromatic ring), (193.0) for (C ,carbonyl of Chalcone CO), (100.0 , 107.3) for (C ,carbons of alkene in chalcone C=CH) , (68.4–73.0) carbons of (N-CH2-CH2-N) ,(CH2=CH2-CH-) carbons of cycle: (23.0-20.1) , 42.1: carbon of (N-CH2-N) ,but derivative{2} appeared many signals at (154.09) for (C , Imine group C=N), (110.1-132.91) for (C ,Aromatic ring), (190.0) for (C ,carbonyl of Chalcone CO) , (102.1 , 104.7) for (C ,carbons of alkene in chalcone C=CH) ,(60.2–73.0) carbons of (N-CH2-CH2-N),(CH2=CH2-CH) carbons of cycle: (19.0-30.9) , 44.1: carbon of (N-CH2-N) ,While derivative{3} appeared many signals at (159.12) for (C , Imine group C=N), (113.4-124.25) for (C , Aromatic ring), (196.4 ) for (C ,carbonyl of Chalcone CO), (101.9 , 104.3) for (C ,carbons of alkene in chalcone C=CH) ,(65.2–71.5) carbons of (N-CH2-CH2-N),(CH2=CH2-CH) carbons of cycle: (15.6-29.3) ,also derivative{4} appeared many signals at (158.7) for (C , Imine group C=N), (113.4 -130.76) for (C , Aromatic ring), (191.5) for (C ,carbonyl of Chalcone CO) ,(100.6 , 105.2) for (C ,carbons of alkene in chalcone C=CH) ,(63.8–78.04) carbons of (N-CH2-CH2-N),(CH2=CH2-CH) carbons of cycle:(16.08-31.21) , 53.81: carbon of (S-CH2-N) ,and the last derivative{5} appeared at (157.22) for (C , Imine group C=N), (117.43-136.08) for (C , Aromatic ring), (188.91) for (C , carbonyl of Chalcone CO) , (101.17 , 103.45) for (C , carbons of alkene in chalcone C=CH) , (69.78–74.27) carbons of (N-CH2-CH2-N),(CH2=CH2-CH) carbons of cycle: (18.66-30.85) , 55.61: carbon of (N-CH2-N).

**Selected Bacteria:**

_Pseudomonas aeruginosa_ is a common encapsulated, Gram-negative, rod-shaped bacterium that canister source of disease in plants and wildlife, involving humans. A species of considerable medical status, _P. aeruginosa_ is a multidrug resistant pathogen renowned for its ubiquity, its intrinsically innovative antibiotic resistance mechanisms, and its connotation with grave infections–hospital-acquired infections like ventilator-associated pneumonia and numerous sepsis syndromes.
Staphylococcus aureus is a Gram-positive, round-shaped bacterium that is a member of the Firmicutes, also it is a usual associate\cite{31,32} of the microbe\cite{Nagham et al 2015.,Intisar and Nagham 2018} of the frame, regularly institute in the upper respiratory tract and on the skin. It is repeatedly positive for catalase and nitrate reduction and is a facultative anaerobe that can cultivate without the requirement for oxygen.

Klebsiella pneumoniae is a Gram-negative, non-motile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium. It seems as a mucoid lactose fermenter on MacConkey agar.

Escherichia coli: also known as (E. coli) is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus Escherichia that is normally originate in the inferior intestine of warm-blooded organisms. The bacterium raises immensely in additional fecal matter below aerobic situations for 3 days, but its facts failure gradually later.

Microbial Resistance Assay\cite{35,36};

Microbial tests for prepared ciprofloxacin derivatives have been screened for their antibacterial activities thru agar via following procedures\cite{35-40} (Nagham et al 2015.,Intisar and Nagham 2018) The test of bacterial inhibition were done at (three concentrations) (10,15,20 micro gram) concentrations in (DMSO) with bacteria:(K. Pneuomona ,S. aureus ,P. aeruginosa ,E- Coli). These bacterial strains were incubated for (24 hr) at temperature (37°C).

The test of resistance of the bacteria\cite{40-44} ,which included kinds of bacteria to curtain the biotic activity of confident derivatives against bacteria.,Tables(1 and 2) appears the diameter of inhibition zone(mm) for vehicles chemical considered towards the bacteria.

**TABLE.1: Inhibition test of derivatives in Concentration (15 micro gram) for (+ gram)**

<table>
<thead>
<tr>
<th>Ciprofloxacin Derivatives</th>
<th>S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Derivative{1}</td>
<td>+++</td>
</tr>
<tr>
<td>Derivative{2}</td>
<td>+++</td>
</tr>
<tr>
<td>Derivative{3}</td>
<td>+++</td>
</tr>
<tr>
<td>Derivative{4}</td>
<td>+++</td>
</tr>
<tr>
<td>Derivative{5}</td>
<td>+++</td>
</tr>
</tbody>
</table>

(+) inhibition(6-9)mm

(++) inhibition(10-14)mm

(+++) inhibition(15-18)mm

**TABLE.2: Inhibition test of derivatives in Concentration (15 micro gram) for (- gram)**

<table>
<thead>
<tr>
<th>Ciprofloxacin Derivatives</th>
<th>K. Pneumona</th>
<th>P. aeruginosa</th>
<th>E- Coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Derivative{1}</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Derivative{2}</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Derivative{3}</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Derivative{4}</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Derivative{5}</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

(+) inhibition(6-9)mm

(++) inhibition(10-14)mm
The grades in this work appeared that the sensitivity of ciprofloxacin derivatives\(^{5,3}\) is higher than other derivatives in the inhibition of kind from selected bacteria, thiadiazole ring, formazan group (N=N-C=N) gave high resistance activity to these derivatives against selected bacteria. Ciprofloxacin derivatives are broadly recycled for the cure of numerous types of bacterial pollutions and infection. General, these antibacterial mediators can be measured safe and well accepted drugs. Relative studies have estimated the practice of quinolones in old and younger inhabitants.

There is no recognized cross-resistance among ciprofloxacin and other modules of antimicrobials. Especially the drug has 100 times higher attraction for bacterial DNA gyrase than for mammalian and prevents bacterial DNA gyrase, an enzyme important for DNA replication. The mechanisms of resistance are known by decreasing their binding affinity to quinolones, decreasing the drugs’ effectiveness.

**Conclusions**

All ciprofloxacin derivatives appeared good resistance towered bacteria and the sensitivity of ciprofloxacin derivatives\(^{5,3}\) is higher than other derivatives in the inhibition of kind from selected bacteria.

**Conflict of Interest:** There is no any Conflict of Interest

**Ethical Clearance:** Ethics committee refer that there is no plagiarism and there is no mistakes or wrong results in this work.

**Source of Funding:** Self funding.

**References**


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Comparison of Surgical Outcomes between Patients Treated with The Harmonic Scalpel and Ligasure Device During Total/Near Total Open Thyroidectomy

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Abstract

Over the last few years many surgeons have begun to utilize the LigaSure device or Harmonic scalpel to perform thyroid surgery. Several papers have demonstrated the benefits of these devices over traditional hand-tying techniques. The purpose of this study was to examine our institution’s experience with the LigaSure device and Harmonic scalpel during thyroid surgery and to compare mean operative times and complications associated with each device. The aim of the study was to compare surgical time, postoperative complications and other parameters between ligasure device and harmonic scalpel in the open thyroidectomy procedure. We choose to examine our institution’s experience with the LigaSure and Harmonic scalpel during total/near total thyroidectomy.

Key Words: Thyroid, Harmonic Scalpel, Ligasure

Introduction

With blood supply from four to five vessels, the thyroid gland represents one of the most vascularized organs of the human body. Its close proximity to neighboring structures, especially the recurrent laryngeal nerves (RLN) and the parathyroid glands, leaves limited room for surgical manipulation. Therefore hemostasis and injury to neighboring structures are of concern (Peter CA and Dirk 2016).[3]

Surgery is one of the standard therapies for numerous thyroid diseases. A total of 80,000 thyroidectomies per year are performed in the United States alone (Bhattacharyya and Fried 2002).[4] The thyroid is the highly vascularized gland and hemostasis is one of the key limiting factors in morbidity and mortality in thyroid surgery.[5] Similar to bleeding, other possible sources of postoperative morbidity include dysphonia and dysphagia due to recurrent and/or superior laryngeal nerve injury, hypocalcemia due to parathyroid ischemia or unintended deprivation, postsurgical hemorrhage, wound infection and postoperative pain. However, the risk of perioperative mortality or major disability[6, 7] is extremely low (Chiang et al 2004., Shindo et al 2000). Harmonic scalpel is manufactures and marketed by Ethicon. The HS is a high power system which works at a frequency of 55.5 KHz or 55,500 vibrations/sec. Dissection by ultrasonic is called ultracision[8,9] . The ultrasound (US) transducer located in the handpiece is composed of piezoelectric crystal sandwiched under pressure among metal cylinders. The US generator converts ultrasonic energy into mechanical[10] energy (Slakey 2008). The sealing of the vessels is achieved due to denatured protein coagulum which occurs due to tamponade and coaptation. It can coagulate[11,12] vessels up to 5 mm(Meurisse et al 2000., Macario et al 2008).

Patients and Method

The study was carried out between 1/10/2015 and 30/8/2017, in which 120 patients having various thyroid diseases were operated in our institution, all were scheduled for total / near total thyroidectomy for different indications they were divided into two groups according to the hemostatic techniques.

One hundred twenty patients were enrolled in this study, 55 patients (54.2%) using ligasure vessel-sealing system (LVSS), 65 (45.8 %) patients using harmonic scalpel (HS). All the operations were performed by surgical teams after consent the patients.
To eliminate confounding factors the following exclusion criteria adopted.

**Those are patients:**
1. with preoperative low serum ca.
2. with vocal cord palsy on preoperative assessment.
3. with need for cervical lymph node dissection.
4. with previous thyroid surgery (recurrence, completion thyroideectomy).
5. with history of neck irradiation.
6. with coagulopathy and bleeding disorders.
7. Pendred ‘s syndrome.

**The study domains were:**
1. Operative time in minutes.
2. Postoperative hoarseness.
3. Post-operative bleeding (drainage volume in ml).
4. Recurrent laryngeal nerve (RLN) status.
5. Total serum calcium level.

**In addition to the baseline pre-operative work up , patients were investigated for:**
1. Thyroid function test (T3, T4, and TSH).
2. Pre-operative indirect laryngoscopy for vocal cords assessment.
3. Pre-operative total serum calcium.
5. Fine needle aspiration for cytology.

All hypothyroid and thyrotoxic patients, including those with Graves' disease, were treated pharmacologically until they became euthyroid (TSH, T3, T4 levels were within normal ranges), after which they were cleared for surgery. Routinely, patients underwent total/near total thyroidectomy for benign thyroid disease, with a focus on recognition and preservation of the laryngeal nerves, along with at least 3 parathyroid glands. The patients had general anesthesia with endotracheal intubation with collar incision 2-3 cm above suprasternal notch. We used ligasure small jaw sealer (Covidien, L 1212 A) (fig.1) was used in one group and for the other group, harmonic scalpel (Ethicon: Johnson & Johnson) for cutting and coagulation where the generator machine can be adjusted from level 1 to 4 to increase cutting speed and decrease coagulation by increasing the blade’s lateral excursion. Closed suction system (Redivac) drain was used to drain post-operative blood over next 24-48 hrs. Vocal cords were checked during recovery by the anesthetist and if any kind of immobility or malfunction was found, the patient was followed up at 1 monthly intervals till 6 months. Patients were assessed for complaint of paresthesia, perioral tingling, examined for chvostek’s sign, trousseau’s sign and Serum calcium was measured during the first and second post-operative days. A level of 8 mg/dl (2mmol/l) was considered as a cutoff value for hypocalcemia. If it was found, the patient would be given calcium and vitamin D3 preparations. Hypocalcemia and RLN palsy were regarded transient if resolved within first 6 months and permanent if they continued after 6 months.

Quantitative domains (serum calcium, operative time and postoperative bleeding volume were expressed as mean ± Standard deviation (SD) and qualitative domains (gender, diagnosis, RLN palsy, transient hoarseness) was given as frequency and percentage. Statistical significance between the two devices for qualitative domains, was assessed using Fischer's Exact test while for quantitative domains, simple descriptive statistic tests were used. The data were entered, coded and analyzed using SPSS version 22 and p-value of 0.05 was considered significant.
Results

A total of 120 patients were enrolled in this study, 95 females (79.2%) & 25 males (20.8%) (Table 1). ♀:♂ (3.8:1). The mean age at surgery 43.89±11.17 (minimum 22 and maximum 66 years). Hemostasis achieved by harmonic device in 65 patients (54.2%) and by LigaSure vessel-sealing system in 55 patients (45.8%). Of the harmonic group 12 out of 65 patients were male, while in ligasure group 13 out of 55 patients were male. Various thyroid pathologies included in our study sample were diagnosis confirmed by histopathological examination (Table 1).

Table 1: Distribution and Frequency Data of Sample

<table>
<thead>
<tr>
<th>Gender</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>95</td>
<td>79.2</td>
</tr>
<tr>
<td>Male</td>
<td>25</td>
<td>20.8</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>100.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Device</th>
<th>Frequency</th>
<th>Percent</th>
<th>♀</th>
<th>♂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harmonic device</td>
<td>65</td>
<td>54.2</td>
<td>53</td>
<td>12</td>
</tr>
<tr>
<td>Ligasure device</td>
<td>55</td>
<td>45.8</td>
<td>42</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>100.0</td>
<td>95</td>
<td>25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple MNG</td>
<td>65</td>
<td>54.2</td>
</tr>
<tr>
<td>Toxic MNG</td>
<td>20</td>
<td>16.7</td>
</tr>
<tr>
<td>Graves disease</td>
<td>11</td>
<td>9.2</td>
</tr>
<tr>
<td>Papillary carcinoma</td>
<td>11</td>
<td>9.2</td>
</tr>
<tr>
<td>Hashimoto’s thyroiditis</td>
<td>8</td>
<td>6.7</td>
</tr>
<tr>
<td>Follicular carcinoma</td>
<td>3</td>
<td>2.5</td>
</tr>
<tr>
<td>Riedels thyroiditis</td>
<td>1</td>
<td>0.8</td>
</tr>
<tr>
<td>Hurthle cell cancer</td>
<td>1</td>
<td>0.8</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>100.0</td>
</tr>
</tbody>
</table>
The patients were matched for age and gender (male, female), no statistically significant difference between the two groups were detected as reported in table (p-value 0.35, 0.51, 0.32 respectively). The mean surgical time with harmonic hemostasis was shorter (56.6 ± 5.8 min) than in LigaSure hemostasis (70.3 ± 5.4 min). Statistically the difference was highly significant (P-value 0.001). When complications rate were examined there was no significant differences in the mean volume of post-operative blood loss between harmonic device (102.8 ± 16.5 ml) and ligasure (101.5 ± 16.4 ml), the p-value was (0.63). The average serum calcium for patients operated with HS was 8.78 and with ligasure 8.81. Postoperative hypocalcemia was observed in (12/65) patients which represent 18.46% of harmonic scalpel group and (11/55) patients which represent 20% of LigaSure group, the result was statistically non significant (p-value 0.78). 4 out of 12 (6.1%) patients continue to have hypocalcaemia beyond 6 months in harmonic group and 3 out of 11 (5.4%) patients in ligasure group (p-value 0.70), (Table 2).

Table 2: Comparison, Demographic data demonstrate no differences of gender and age distribution for use of each device

<table>
<thead>
<tr>
<th>Device</th>
<th>Harmonic device</th>
<th>LigaSure device</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>53</td>
<td>42</td>
<td>0.32</td>
</tr>
<tr>
<td>Male</td>
<td>12</td>
<td>13</td>
<td>0.51</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Device</th>
<th>Harmonic device</th>
<th>LigaSure device</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>41.9(10.9)</td>
<td>46.3(10.9)</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Comparison of operative time between the use of two hemostasis devices

<table>
<thead>
<tr>
<th>Device</th>
<th>Harmonic device</th>
<th>LigaSure device</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operative time in min (±SD)</td>
<td>56.6(5.8)</td>
<td>70.3(5.4)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Differences in Blood loss between the use of two devices

<table>
<thead>
<tr>
<th>Device</th>
<th>Harmonic device</th>
<th>LigaSure device</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood loss in ml (±SD)</td>
<td>102.8(16.5)</td>
<td>101.5(16.4)</td>
<td>0.63</td>
</tr>
</tbody>
</table>

The differences in frequency of hypocalcaemia in each device

<table>
<thead>
<tr>
<th>Device</th>
<th>Harmonic device</th>
<th>LigaSure device</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypocalcaemia</td>
<td>12</td>
<td>11</td>
<td>0.78</td>
</tr>
</tbody>
</table>

Frequency of permanent Hypocalcaemia in devices used

<table>
<thead>
<tr>
<th>Device</th>
<th>Harmonic device</th>
<th>LigaSure device</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypocalcaemia</td>
<td>4</td>
<td>3</td>
<td>0.70</td>
</tr>
</tbody>
</table>
Twenty three patients experienced postoperative hoarseness from the total (120) patients. Postoperative hoarseness was indifferent between the two devices, with (12/65) patients of harmonic group (18.46%) experiencing hoarseness compared to (11/55) patients (20%) in ligaSure group (p-value 0.83). Of the 23 patients with hoarseness, 5 patients (4.16%) reported permanent RLN palsy (beyond 6 months) in harmonic group 4/65 (6.15%) while in ligaSure group 1/55 (1.81%) which was statistically significant, p-value (0.001).

Table (3): frequency of postoperative hoarseness in our study (total)

<table>
<thead>
<tr>
<th>postoperative hoarseness</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>97</td>
<td>80.8</td>
</tr>
<tr>
<td>Yes</td>
<td>23</td>
<td>19.2</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>100.0</td>
</tr>
</tbody>
</table>

The differences in frequency of postoperative hoarseness (in each device)

<table>
<thead>
<tr>
<th>Device/operative hoarseness</th>
<th>Harmonic device</th>
<th>Ligasure device</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>hoarseness</td>
<td>12</td>
<td>11</td>
<td>0.83</td>
</tr>
</tbody>
</table>

Frequency of permanent RLN palsy (total)

<table>
<thead>
<tr>
<th>RLN palsy</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO</td>
<td>115</td>
<td>95.8</td>
</tr>
<tr>
<td>Yes</td>
<td>5</td>
<td>4.16</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>100.0</td>
</tr>
</tbody>
</table>

The differences in frequency of permanent RLN injury (in each device)

<table>
<thead>
<tr>
<th>RLN palsy/Device</th>
<th>harmonic device</th>
<th>Ligasure device</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RLN palsy</td>
<td>4</td>
<td>1</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Discussion

Total thyroidectomy is a very delicate procedure requiring extreme care to avoid injury to neighboring anatomical structures. Strict hemostatic control is fundamental for limiting the risk of serious complications, including recurrent laryngeal nerve and parathyroid gland injuries, and for decreasing the incidence of other postoperative complications.  

Given the importance of optimizing hemostatic control, the use of devices to dissect and close vessels during thyroidectomy is not uncommon. The aim of study was to provide a direct comparison of clinical outcomes associated with each of two hemostatic techniques used for total thyroidectomy: LigaSure, and Harmonic device.

The mean surgical time in total thyroidectomy with harmonic hemostasis was shorter (56.6±5.8) than in ligaSure hemostasis (70.3±5.4). Statistically the difference was highly significant (P-value 0.001). Our result consistent with that of Arun Upadhyaya et al who reported that the surgical time was significantly decreased in the HS group compared with the LS instrument group, and in agreement with that of McNally et al which was a retrospective analysis of prospectively maintained database, they compare the effectiveness and safety of ultrasonic dissection (UD) and electronic vessel sealing (EVS) in patients undergoing thyroidectomy between January 1, 2007 and January 25, 2008.
found significantly decreased operative time with UD versus EVS. Contin et al published\[14\] a systematic review and meta-analysis comparing HS, LS and a conventional technique in 2013. The study compared HS and LS with the conventional technique, and HS and LS separately. The study concluded that HS and LS were significantly different when compared to conventional hemostasis in terms of shorter surgical time. It also concluded that HS was faster in comparison to LS. This was contrary to study by Kwak HY et al to evaluate the safety and efficacy of thyroidectomy using the Harmonic scalpel (HS) or the LigaSure Precise (LS) instrument in 320 patients (HS group, N=164; LS instrument group, N=156) who demonstrate no statistically significant differences in the operative times between the two techniques. Our result was inconsistent with that of Macario et al who\[18\] reported that the operation time of LigaSure electro surgical bipolar sealing system is less than that of the ultrasonic device. The HS is unique in the fact that it combines hemostasis and cutting in a single instrument, thus avoiding the loss of time when associated with the manipulation of several instruments. By contrast, the LS instrument can only be used for hemostasis, and sectioning was performed with another instruments such as scissors after withdrawal of the device. The shorter operative time may be attributed to the fact that LS is a more time-consuming multiple-sealing approach at the same vessel but HS divides the tissue at the same time of coagulation. Regarding RLN palsy, we found a statistically significant differences between\[15\] the two techniques where incidence was higher with harmonic, which inconsistent with the fact that LigaSure had a range of 2 to 3 mm for thermal injury; the Harmonic scalpel had safety measure of 2 mm for thermal injury. Our result contrary to many studies which reveal that HS has less damage to nearby vital structures like the RLN and the parathyroid glands (Obonna and Mishra 2014)[17].

On 2016 Dequanter et al investigated the extent of heat injury, ultrasonic dissection did not cause any immediate damage of the nerve even close to the RLN (1 mm away from the RLN). They concluded that the use of harmonic scalpel for thyroid surgery is safe for the surrounding structures (nerves). Careful tissue applications of the device near the RLN and the parathyroid glands (Obonna and Mishra 2014)[17].

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Conclusions

In this study, there was no difference in the rate of complications between the two devices except for RLN palsy. However, the use of the Harmonic
scalpel significantly decreased operative time for thyroidectomies when compared to the LigaSure device. They are safe and effective advantageous for sealing medium-sized vessels, however a scrupulous and careful care of the surgical technique and hemostasis should always be observed.

Conflict of Interest: There is no any Conflict of Interest

Ethical Clearance: Ethics committee refer that there is no plagiarism and there is no mistakes or wrong results in this work.

Source of Funding: Self funding.

References

Safe Concentration of Benzene Exposure Based on Safe Human Dose of Workers in The Paint Manufacturing Industry Sidoarjo, East Java, Indonesia

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1Student Departement of Occupational Health and Safety, Public Health Faculty, Airlangga University, Surabaya, 2Department of Occupational Health and Safety, Faculty of Public Health, Airlangga University, Indonesia

Abstract

Background: Manufacture of paints using benzene in large quantities in the products they produce. Workers in the paint industry may face an increased risk of high health complications. The purpose of this study was to determine the levels of safe concentration of benzene for workers in the Paint Manufacturing Industry in Sidoarjo East Java, Indonesia. Methods: This research was conducted using a cross sectional method. The sample in this study were 24 respondents. The variables that became the data of this study included benzene concentration (C) time of daily exposure (tE), frequency of annual exposure (fE), duration of exposure (Dt), height (cm), weight (kg), age, respiration rate (BR), body surface area, weight of white rat, body surface of white rat, highest dose of toxin without effect on experimental animals (NOAEL), Km factor in animal (Animal Km), Km factor on workers (Human Km), safe limits for toxin doses for workers (SHD), and benzene concentrations in safe air for workers (C is safe). Data analysis in this study was carried out by using quantitative data analysis manually to determine the safe concentration of benzene for workers in the Paint Manufacturing Industry in Sidoarjo East Java, Indonesia. Results: Based on the results of measurements carried out, the concentration of benzene in the work environment of the Sidoarjo Paint Industry in the mixing solvent section is 2 ppm and in the packing section is 0.4 ppm, the safe concentration is 0.028 ppm. Conclusion: The recommended safe concentration of benzene exposure is 0.028 ppm. The safe concentration value exceeds the set value of 0.009 ppm daily for acute effects and 0.003 ppm daily for chronic effects, so control measures are needed to protect against the adverse effects of benzene on the health of workers. Control recommendations are to provide a good exhaust ventilation system at the paint manufacturing.

Keywords: Benzene Exposure, Safe Concentration, Paint Manufacturing

Introduction

The Paint manufacturing industry uses benzene in the products they produce in large quantities. Workers in the paint industry may overcome high health problems. Paint manufacturers usually use three main components in the manufacture of their products consisting of coloring agents, binding agents, and solvents that allow other components to remain in liquid form. Solvents are widely used in industries such as benzene. Using benzene as a solvent agent allows manufacturers to use materials that are cheaper and easier to obtain in their products to make high quality, inexpensive effective products to obtain. Individuals who work in cat-making facilities, either make products or service equipment used to produce products, according to the highest needs of long-term Benzene. Other individuals working in facilities are also at risk because benzene can enter the air during the manufacturing process.

Many industrial sectors use organic solvents. Organic solvents such as benzene can result in damage to the nervous system (central and peripheral), kidney damage, and liver, adverse reproductive effects, such as sperm changes and infertility, skin lesions, and cancer. In paint production, solvent vapor is removed during the manufacturing process. If these emissions are left uncontrolled, high concentrations of organic solvents can accumulate in the work area, which endangers the health and safety of workers. Removing volatile organic
solvents into the atmosphere can cause increased tropospheric ozone levels, pollutants that cause negative health effects on the human lung system. Appropriate exhaust ventilation applications are used to remove contaminants produced by operations to maintain a healthy work environment.

The Threshold Limit Value (TLV) of benzene chemicals in the workplace is the maximum allowable value is 0.5 ppm and is certainly included as a carcinogenic group in humans (A1 = Confirmed Human Carcinogen). The recommended exposure limit for 8 working hours is 0.1 ppm. In Indonesia, TLV benzene is 0.5 ppm. Whereas based on the Minimum Risk Level (MRL) set for benzene is 0.009 ppm daily can have an acute effect and 0.003 ppm every day has a chronic effect. The aim of this study was to determine the level of safe concentration of benzene for workers in the Paint Manufacturing in Sidoarjo, East Java, Indonesia.

**Methods and Materials**

This research was conducted in the Paint Manufacturing in Sidoarjo, East Java, Indonesia. This research was conducted using a cross sectional method. The sample in this study were 24 respondents. Data was taken including measuring the concentration of benzene, filling in questionnaires and interviewing workers, while also collecting primary data in experimental animals namely the weight of white rats. Data on the concentration of benzene in the air was obtained by direct measurement using Coconut shell charcoal and analyzed by Gas Chromatography (GC), referring to the NIOSH 1501 method.

The variables in this study include benzene concentration (C) daily exposure time (tE), frequency of annual exposure (fE), duration of exposure (Dt), height (cm), weight (kg), age, rate respiration (BR), body surface area of workers, weight of white mice, body surface area of rats, highest dose of toxin without effect on experimental animals (NOAEL), Km factor in animals (Animal Km), Km factor in workers (Human Km), safe limit of toxin dosage for workers (SHD), and benzene concentration in safe air for workers (safe consentration).

Based on the data of white rat body weight, the body surface of the white mouse can be calculated using the following formula:

\[ BSA_{Animal} = 0.09 W^{0.67} \]

**Description:**

BSA : Body Surface Area (m²)
W : Body Weight (kg)

Based on data weight and height of workers, the body surface area and the rate of respiration of workers can be calculated using the following formula.

\[ BSA = \sqrt[3]{W.h/3600} \]

**Description:**

BSA : Body Surface Area (m2)
W : Body Weight (kg)
h : Height (cm)

Respiratory rate of workers

\[ BR = 5.3 \ln W - 6.9 / 24 \]

**Description:**

BR : Breathing Rate (m³/jam)
W : Body Weight (kg)

Determination of safe limits of toxic doses for workers begins first by calculating Animal Km and Human Km.

Animal Km:

\[ \text{Animal Km} = \frac{W_{animal}}{BSA_{animal}} \]

**Description:**

Animal Km : Km factor in animals
W : Experimental animal weight (white mouse)
BSA : Body Surface Area of experimental animals (White mice)

Human Km:

\[ \text{Human Km} = \frac{W_{human}}{BSA_{human}} \]

**Description:**
Human Km : Km factor in humans / workers
W : Worker weight
BSA : Body Surface Area of worker

One of the objectives of research activities in the field of toxicology is to be able to evaluate the safety of a substance. To determine the safe limit of the concentration of a chemical begins with a toxicity test determining the highest dose without causing effects on experimental animals or No Observed Adverse Effect Level (NOAEL).

The safe limit of dosage of toxins for workers or Safe Human Dose (SHD) is found to begin by using the following formula:

\[ SHD = \frac{\text{Animal Km}}{\text{Human Km}} \text{ NOAEL} \]

Description:
SHD : Safe Human Dose (mg/kg)
Animal Km : Km factor in animals
Human Km : Km factor in humans / workers

Determining the safe limit of benzene concentration in the work environment uses the following formula:

\[ \text{Safe Concentration} = \frac{(SHD)(W)}{(\delta)(BR)(t)} \text{g/m}^3 \]

To convert units of mg / m3 to ppm the following formula is used:

\[ \text{Safe Concentration} = \frac{\# \text{ mg/m}^3 \times 24.5 \text{ ppm}}{(MW)} \]

Description:
Safe Concentration : toxic concentration in safe air for workers (mg/m3)
SHD : Safe Human Dose (mg/kg)
W : Body Weight (kg)
\( \delta \) : % of substances absorbed by the lungs
BR : Human respiratory rate (m3/jam)
t : Working time (hour)

MW : Molecular Weight

FINDINGS

Distribution of Characteristics of Workers

Figure 1. Distribution of Characteristics of Workers

Figure 1. Obtained average weight data (W) of respondents is 60.71 kg, the average height (H) of respondents is 159 cm, the average length of work (Dt) of respondents is 13 years. The duration of working a day (tE) is 7 hours, the number of workdays in a year (fE) is 240. The results of the analysis of calculation of body surface area and worker respiratory rate according to table 2 show that the average body surface area of workers (BSA) is 1.63 m2 and the average respiration rate of workers (BR) is 0.62 m3 / hour.

Distribution of benzene concentration in the workplace

Tabel 1. Distribution of benzene concentration in the workplace

<table>
<thead>
<tr>
<th>Location</th>
<th>C (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixing Solvent</td>
<td>2 ppm</td>
</tr>
<tr>
<td>Packing</td>
<td>0.4 ppm</td>
</tr>
</tbody>
</table>

Tabel 1. Based on the results of the measurements made, the concentration of benzene in the work environment of the Paint Manufacturing Industry in Sidoarjo at the mixing solvent section is 2 ppm and in the packing section is 0.4 ppm.
Animal Km

Tabel 2. Calculation Results of Animal Km (White Rat)

<table>
<thead>
<tr>
<th>Object (White Rats)</th>
<th>W (kg)</th>
<th>BSA (m²)</th>
<th>Animal KM = W/BSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0,1405</td>
<td>0,024165</td>
<td>5,814194082</td>
</tr>
<tr>
<td>2</td>
<td>0,1405</td>
<td>0,024165</td>
<td>5,814194082</td>
</tr>
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<td>5,80041580</td>
</tr>
<tr>
<td>6</td>
<td>0,1415</td>
<td>0,024165</td>
<td>5,855576247</td>
</tr>
<tr>
<td>Total</td>
<td>0,7025</td>
<td>0,120826</td>
<td>29,07063198</td>
</tr>
<tr>
<td>Average</td>
<td>0,141</td>
<td>0,02</td>
<td>5,81</td>
</tr>
</tbody>
</table>

Table 2. The results of the Animal Km calculation are shown in table 2, with Animal Km averages of 5.81.

Human Km

Figure 2. Results of Human Km Calculation on Workers

Figure 2. The results of the Human Km calculation are shown in table 2, with a Human Km average of 36.94.

No Observed Adverse Effect Level (NOAEL)

Benzene NOAEL is 3.0 mg / m³ or equivalent to 0.022 mg / kg obtained from the calculation of the formula as follows:\(^9\):

\[
\text{NOAEL} = \frac{3 \times 0.002}{3 \times 0.003 \times 8} = 0.003 \text{mg/kg}
\]

Safe Human Dose

Based on the formula from Shaw et al., The calculation of SHD obtained from the NOAEL value, the average animal Km, and the average human Km is 0.003 mg / kg based on the calculation below:

\[
\text{SHD} = \frac{0.022 \text{mg/kg}}{\frac{5.81}{36.94}} = 0.003 \text{mg/kg}
\]

Safe Concentration of Benzene

Calculation of safe concentration of benzene in the Paint Manufacturing Industry in Sidoarjo is 0.028 ppm obtained from the calculation below:

\[
\text{Safe Concentration} = \frac{0.003 \times 60.71}{(50\%)(0.6)(7)} = 0.008 \text{mg/m}^3
\]

\[
\text{Safe Concentration} = \frac{0.008 \text{mg/m}^3 \times 24.45}{78.11} = 0.028 \text{ppm}
\]

Discussion

Concentrations of Benzene Exposure

Based on the results of the measurements made, the concentration of benzene in the work environment of the Paint Manufacturing Industry in Sidoarjo at the mixing solvent section is 2 ppm and in the packing section is 0.4 ppm. The benzene concentration in the mixing solvent section is above the Threshold Value (NAB) while the benzene concentration in the packing section is below the Threshold Value (NAB) of 0.5 ppm\(^7\). However, the concentration of benzene is above the Minimum Risk Level (MRL), the level of exposure to benzene inhalation is determined namely for acute exposure (≤14 days) = 0.009 ppm, moderate exposure (15-364 days) = 0.006 ppm, and chronic exposure (≥365 days) = 0.003 ppm\(^8\).

Safe Concentration to Workers

Research shows that safe concentration for workers in the Sidoarjo paint industry is 0.028 ppm. Benzene is carcinogenic in humans and concentrated air benzene is associated with excessive lifetime risk resulting in leukemia\(^10\). The minimum risk level of benzene at a concentration of 0.009 ppm has been reduced for the duration of exposure to acute inhalation (14 days or less), the minimum risk level of 0.006 ppm benzene concentration has been lowered for medium duration inhalation exposure (15-364 days) and minimum risk level of benzene 0.003 ppm reduced for chronic duration inhalation exposure (364 days or more)\(^8\).

Benzene can enter the body through the digestive tract, lungs or skin (EPA). When exposed to high
levels of benzene, about half of the benzene inhaled passes through the lining of the lungs and enters the bloodstream. With high concentrations of benzene in short exposures in the range of hundreds of ppm can cause confusion, tremor, headaches, and unconsciousness. While continuous exposure to low concentrations can cause blood-related diseases such as excessive bleeding, anemia, and decreased immune response. In addition, benzene can also cause acute blood cancer or myeloid anemia because it is carcinogenic. This research can contribute as one of the studies on the topic of risk assessment which is still limited in Indonesia, especially focusing on the standard benzene concentration in the paint manufacturing industry. This research is also limited in a number of sampling, requiring improvements in method design (not only cross-sectional but also in case-control, cohort design or experiments), and further research is needed to recognize the risk assessment of benzene in the paint industry. Control recommendations are to provide a good exhaust ventilation system at the paint manufacturing plant, consume CYP2E1 enzymes contained in beef liver which serve to reduce the level of benzene in the body, use of appropriate Personal Protective Equipment.

**Conclusion**

Workers in the Mixing Solvent section at the Indonesian Paint Industry in Sidoarjo showed benzene exposure concentrations of 2 ppm, which was above the threshold value of 0.5 ppm according to the Minister of Manpower and Transmigration Regulation No. 13 of 2011, included in the health risk category. Unsafe concentration for workers. The recommended safe concentration of benzene exposure is 0.028 ppm. The safe concentration value if according to the Minimum Risk Level (MRL) exceeds that which is set at 0.009 ppm daily for acute effects and 0.003 ppm daily for chronic effects, so that control efforts are needed to be protected from the adverse effects of benzene on the health of workers. Control recommendations are to provide a good exhaust ventilation system in the paint manufacturing industry.

**Conflicts of Interest**: All authors have no conflict interest to declare.

**Source of Funding**: The source of the research cost from self.

**Ethical Clearance**: The study was approved by the Ethics Committee Faculty of Public Health, Airlangga University.

**Reference**

1. Ridgwaya P, Nixon T, Leach J. Occupational exposure to organic solvents and long-term nervous system damage detectable by brain imaging, neurophysiology or histopathology. Food Chem Toxicol. 2003;
2. NIOSH. Occupational Diseases - A Guide to their Recognition. 1977;
5. ACGIH. Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices. Cincinnati: American Conference of Governmental Industrial Hygienists; 2014.
Determination of the Health Costs of Effective Detoxification Based on Effective Doses of Benzene Exposure To workers in oil and gas mining companies, Central Java, Indonesia

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Abstract

Benzene is a natural component of crude oil and gasoline. Benzene occurs naturally in crude oil at levels of up to 4 g /l and benzene has been classified as a group carcinogen. This research was conducted in oil and gas mining companies, West Java, Indonesia. This research was conducted using a cross sectional method. The sample in this study were 30 respondents. The variables that became the data of this study included benzene concentration (C) daily exposure time (tE), frequency of annual exposure (fE), duration of exposure (Dt), height, weight, age, respiration rate (BR), and urine of workers.

The results showed an average measurement of benzene concentration in oil and gas mining companies, West Java, Indonesia on the KPC portion of 0.1 ppm and in the laboratory section at 0.2 ppm, which means it is still below the Threshold Value (NAB) which is equal to 0.5 ppm5. Obtained the highest effective dose to be consumed by respondents in the KPC unit for beef liver is 6x10 -4 kg at a cost of IDR 33,31046813, chicken 3x10-4 kg at a cost of IDR 13,1251,9985, and avocado is 4x10 -6 kg at a cost of IDR 0,16206628, while the highest effective dose to be consumed by respondents in the laboratory unit for beef liver is 1x10-2 kg at a cost of IDR 12,14687477, chicken 6x10-4 kg at a cost of IDR 22,14504841, and avocado is 6x10-6 kg at a cost of IDR 0, 27344083. Control recommendations are to adopt a lifestyle that can minimize exposure to poisons and improve detoxification pathways.

Keywords: Costs Effective, Dose Effective , Detoxification, Benzene

Introduction

Benzene is a natural component of crude oil and gasoline. Benzene exposure results in health-related effects and diseases such as cancer. Exposure can occur as a result of the use of petroleum products which contain benzene in it. Benzene occurs naturally in crude oil at levels of up to 4 g /l. These activities include the processing of petroleum products, coal coke, the production of toluene, xylene, and other aromatic compounds. The presence of benzene in gasoline and as a widely used industrial solvent can result in significant exposure and broad emissions to the environment1.

Long-term exposure to benzene can cause leukemia, and benzene has been classified as a group 1 carcinogen2. The dominant pathway for exposure to benzene in humans through inhalation. Detoxification is a process to remove these persistent and potentially dangerous chemicals from the human body for disease prevention and health recovery3.

The body is an extraordinary system that has an innate mechanism to convert harmful substances into non-toxic compounds that can be removed from the body. This detoxification process, called the detoxification metabolic pathway, involves a series of complex biochemical reactions in cells that are just beginning to be understood by modern science. Briefly, there are two phases in this metabolic pathway. In phase I, a group of enzymes collectively known as cytochrome P450 is responsible for the biotransformation of poisons which are mostly fat-soluble into molecules that are more water-soluble.4 It should be noted that intermediates produced in phase I can be more toxic than original poisons because they are more reactive and can increase oxidative pressure in cells. These substances must be
quickly processed through phase II reactions to prevent damage to cells.

In phase II, various conjugate reactions that differ between a second series of enzymes called conjugates and toxic intermediates from phase I occur. These conjugations attach molecules such as glucuronic acid, sulfate, glutathione, glycine, taurine, or methyl groups to toxic intermediates to make them more soluble in water and unreactive, effectively “detoxifying” these toxic intermediates. These poisons which are converted biotransformation through two phases can then be removed from the cell and released through bile (into feces), urine, or sweat. While the human body has the innate ability to detox, there are many factors that can affect the activity of enzymes involved in this process. Diet, lifestyle, environment, genetics, age, gender, disease, and influence of drugs. Among these factors, there are things that can be controlled, namely the diet. The purpose of this study was to determine the effective health costs in accordance with the effective dosage of benzene and the types of foods that can detoxify benzene in the blood of workers in oil and gas mining companies, West Java, Indonesia, including beef liver, chicken and avocado. environment, while maintaining optimal detoxification capabilities through diet and lifestyle to prevent disease.

**Methods and Materials**

This research was conducted in oil and gas mining companies, West Java, Indonesia. This research was conducted using a cross sectional method. Urine examination to determine levels of benzene exposure to workers. Workers who are sampled must meet the specified criteria (Inclusion). The inclusion criteria that must be fulfilled are the age of the worker > 20 years and the working period > 5 years. The sample in this study were 30 respondents namely 15 people in the Laboratory section and 15 people in the KPC section.

Benzene inhalation sampling was carried out using a personal sampling pump that charcoal tubes had been installed which referred to NIOSH 1501 and sampling and measurement of phenol levels in workers’ urine was carried out based on NIOSH 8305. Measurements of environmental physics parameters were carried out to know the working environment conditions and were used in calculating exposure concentrations benzene.

The benzene dose measured at the time of the study was an intake through inhalation. Because this study will know the chronic effects of benzene exposure, the value of Chronic Daily Intake (CDI) will be calculated.

Then, look for intake (formula) with the formula as below:

\[
\text{Intake} = \frac{C \times \text{IR} \times tE \times fE \times Dt}{Wb \times 70 \times 365}
\]

\[
C = \text{benzene concentration (mg / ml)}
\]

\[
\text{IR} = \text{Inhalation rate (m\(^3\)/days)},
\]

\[
tE = \text{work time / day (hours)}
\]

\[
fE = \text{work time / week (days)}
\]

\[
Dt = \text{working time (years)}
\]

\[
Wb = \text{weight (kg)}
\]

Calculate the effective dose per day with the results of calculating the intake above, using the formula below:

\[
\text{Effective dose of food} = \frac{\text{intake toxin/day} \times \text{Mr toxin/food}}{\text{Mr toxin}}
\]

The last is to calculate the cost of intake of each food using the formula as below:

\[
\text{Cost of intake} = \text{Effective dose of food} \times \text{price per Kg of food}
\]

**Information (in Indonesia):**

**Tabel 1. Cost in Indonesia**

<table>
<thead>
<tr>
<th>No.</th>
<th>Ingredient</th>
<th>Weight</th>
<th>Price (IDR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Beef liver</td>
<td>1 kg</td>
<td>50000</td>
</tr>
<tr>
<td>2</td>
<td>Chicken</td>
<td>1 kg</td>
<td>35000</td>
</tr>
<tr>
<td>3</td>
<td>Avocado</td>
<td>1 kg</td>
<td>45000</td>
</tr>
</tbody>
</table>
RESULT

Distribution of Characteristics of Workers

Figure 1. Distribution of Characteristics of Workers

Figure 1. Obtained the average weight data (W) of respondents is 64 kg, the average height (H) of respondents is 165 cm, the average length of work (Dt) of respondents is 5 years. The duration of work in a day is 8 hours both for workers in KCP units and laboratory workers and the number of working days in a year (Dt) is 274.

Distribution of benzene concentration in the workplace

Figure 2. Distribution of benzene concentration in the workplace

Figure 2. Obtained results The average concentration of benzene in oil and gas mining companies, West Java, Indonesia in the KPC section is 0.191 mg / m³ or 0.1 ppm and in the laboratory part is 0.466 mg / m³ or 0.2 ppm. Based on the Minister of Manpower and Transmigration Regulation No. PER.13 / MEN / X / 2011 of 2011 concerning the Threshold Value of Physical and Chemical Factors in the Workplace, benzene NAB recommended to prevent the occurrence of health impacts is 0.5 ppm.

Comparison Weight and Benzene Concentration

Figure 3. Comparison Weight and Benzene concentration

Figure 3. The highest benzene concentration in the KPC unit was 0.2 ppm (respondent 13) with a body weight of 63 kg, while the smallest benzene concentration in the KCP unit was 0.0013 ppm with a body weight of 68 kg (respondent 7). The biggest weight in the Kcp unit respondents was 68 kg (respondents 7 and 12), while the smallest was 60 kg. The highest concentration in laboratory units was 0.3 ppm (respondents 17) with a body weight of 67, while the lowest was 0.21 ppm (respondents 46-51). the largest weight in laboratory units was 68 (respondents 20 and 26), while the smallest was 60 kg (respondents 18 and 23).

Comparison of Benzene Concentrations with Effective Doses and Effective Prices
<table>
<thead>
<tr>
<th>No.</th>
<th>Unit</th>
<th>C (mg/m³)</th>
<th>effective dose of beef liver (kg)</th>
<th>effective dose of Chicken (kg)</th>
<th>effective dose of avocado (kg)</th>
<th>Cost beef liver</th>
<th>Cost chicken</th>
<th>Cost avocado</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>KPC</td>
<td>0.483</td>
<td>0.00066621</td>
<td>0.00037501</td>
<td>0.00000360</td>
<td>33,31046813</td>
<td>13,12519985</td>
<td>0.16206628</td>
</tr>
<tr>
<td>2</td>
<td>KPC</td>
<td>0.004</td>
<td>0.000000509</td>
<td>0.00000287</td>
<td>0.00000003</td>
<td>0.25464285</td>
<td>0.10033598</td>
<td>0.00123892</td>
</tr>
<tr>
<td>3</td>
<td>KPC</td>
<td>0.084</td>
<td>0.00011586</td>
<td>0.00006522</td>
<td>0.00000063</td>
<td>5.79312489</td>
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</tr>
<tr>
<td>4</td>
<td>KPC</td>
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<td>0.00016677</td>
<td>0.00009387</td>
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<td>8.33858886</td>
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<tr>
<td>5</td>
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<td>0.00000371</td>
<td>0.00000209</td>
<td>0.00000002</td>
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<tr>
<td>6</td>
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<td>0.00000600</td>
<td>0.00000338</td>
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<tr>
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<td>0.00000243</td>
<td>0.00000137</td>
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</tr>
<tr>
<td>8</td>
<td>KPC</td>
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<td>0.00027501</td>
<td>0.00015480</td>
<td>0.00001499</td>
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<tr>
<td>9</td>
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<td>0.00011403</td>
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<tr>
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<td>KPC</td>
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<td>0.00009931</td>
<td>0.00005590</td>
<td>0.0000054</td>
<td>4.96553562</td>
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<td>0.00037537</td>
<td>0.00021129</td>
<td>0.0000203</td>
<td>18,76854238</td>
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<tr>
<td>12</td>
<td>KPC</td>
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<td>0.00058175</td>
<td>0.00032746</td>
<td>0.0000314</td>
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<tr>
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<td>0.00063317</td>
<td>0.00035641</td>
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<tr>
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<td>KPC</td>
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<tr>
<td>17</td>
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<td>0.00063272</td>
<td>0.0000608</td>
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</tr>
<tr>
<td>18</td>
<td>Lab</td>
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<td>0.00041150</td>
<td>0.0000395</td>
<td>36,55185944</td>
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<tr>
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<td>0.00032962</td>
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<td>14,96026758</td>
<td>5,89473858</td>
<td>0.07278657</td>
</tr>
</tbody>
</table>
Table 2. Obtained the highest effective dose that must be consumed by respondents in the KPC unit for beef liver is $6 \times 10^{-4}$ kg at a cost of IDR 33,31046813, chicken $3 \times 10^{-4}$ kg at a cost of IDR 13,12519985, and avocado is $4 \times 10^{-6}$ kg at a cost of IDR 0.16206628, while the highest effective dose to be consumed by respondents in the Laboratory unit for beef liver is $1 \times 10^{-2}$ kg at a cost of IDR 12,14687477, chicken $6 \times 10^{-4}$ kg at a cost of IDR 22,14504841, and avocado is $6 \times 10^{-6}$ kg at a cost IDR 0.27344083.

Discussion

Threshold Limit Value (TLV), Weight, and Benzene Concentration

The results showed an average measurement of benzene concentration in oil and gas mining companies, West Java, Indonesia on the KPC portion of 0.1 ppm and in the laboratory section at 0.2 ppm, which means it is still below the Threshold Value (NAB) which is equal to 0.5 ppm\(^5\). The comparative diagram analysis between the concentration of benzene and the weight of the respondents showed that the respondents with the greatest weight did not have the highest concentration of benzene, and it was similar to the respondents with the smallest weight not having the lowest toluene concentration. This research is not in accordance with Mukono’s research, that BTX has a mass of small molecules that are easily soluble in fat\(^6\). It is assumed that toxins with high solubility in fat (adipose) show low concentration in the body. This can be considered a protection mechanism. So, it was concluded that there was low benzene toxicity in obese people rather than lean people.

Detoxification of Benzene by Foods Rich in CYP2E1 Enzyme, Sulfation, and Glutation

YP2E1, Sulfation, and glutathione are enzymes that work in stage 2 of biotransformation\(^7\). Sulfation is one of a number of liver detoxification pathways, specifically phase II detoxification. Detoxification sulfate poison, it is a powerful antioxidant compound and detoxifying agent that is produced in the cytoplasm of every cell of the human body\(^8,9\). Antioxidant enzymes related to glutathione are involved in the metabolism and detoxification of cytotoxic and carcinogenic compounds and reactive oxygen species \(^10\).

The best approach to detoxification must be to adopt a complete, healthy, complete, complete diet \(^11\). Various fruits and vegetables, best for including vegetables, coriander, garlic, and citrus fruits, fats and oils with medium - Chain fatty acids (Avocados, coconuts, olive oil), Proteins from lean meat, eggs, and vegetable sources (for example tofu, tempeh), complex carbohydrates from seeds such as brown rice, quinoa, millet, soba, legumes such as peas, beans, and lentils, which are rich in soluble and insoluble fiber.

Phase 1 and 2 of the detox system are complex processes and vegetables, fruits, grains, soybeans, garlic, rooibos tea and turmeric have been reported to maintain and support the detoxification phase that occurs in the liver, making it easier to work\(^7\). The two additional nutrients needed by the liver are zinc and choline. Zinc, which can be found in food and contains the most pork, crab, beef and oysters, has been shown to help detoxify the body from alcohol. Choline is needed to make phosphatidylcholine which is used by the liver to produce VLDL cholesterol types. Choline can be found in wheat germ, chicken, beef liver, and eggs\(^12\).

Avocados contain various healthy nutrients such as vitamin A, vitamin C, vitamin E, vitamin K1, folate, vitamin B-6, niacin, pantothenic acid, and bioactive phytochemicals, such as carotenoids, terpenoids, and D-mannoheptulose, which have been reported to help fight cancer\(^13\). Avocados are rich in monounsaturated fatty acids (MUFA), which increase the bioavailability (absorption) of nutrients and phytochemicals. A study published in the American Journal of Clinical Nutrition, consuming high amounts of monosaturated fatty acids was found to reduce LDL cholesterol (‘bad’ cholesterol) by 14% and reduce the risk of cardiovascular disease\(^14\). Avocados also contain glutathione, an important factor in protecting against toxicity and disease\(^15\).

Conclusion

Intake of foods containing the enzyme CYP2E1 (beef liver), sulfation (chicken), and glutathione (avocado) is expected to increase detoxification of benzene. Each individual has a different amount of cost per individual. This depends on the effective dose, benzene concentration, weight, and duration of work. In addition to diet, adopting a lifestyle that can minimize exposure to poisons and improve detoxification pathways is equally important. These lifestyle components include: Avoid environmental toxins, such as heavy metals, persistent organic pollutants, and electromagnetic radiation, mobilize and eliminate toxins through weight loss, use
of saunas, exercise, and chelating supplements such as Chlorella. Maintain optimal intestinal health through diet and probiotics, drink enough water to maintain optimal hydration, reduce emotional stress and maintain healthy relationships, ensure adequate sleep and relaxation.

**Conflicts of Interest**: All authors have no conflict interest to declare.

**Source of Funding**: The source of the research cost from self.

**Ethical Clearance**: The study was approved by the Ethics Committee Faculty of Public Health, Airlangga University.

**Reference**

Relationship of Hot Work Climate with Employee Blood Pressure in Binat Installation, Dr. Sardjito

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Abstract
Blood pressure in each individual varies, and depends on age and daily activities such as when blood pressure activity will rise and when the remaining blood pressure will drop. Laundry installations are workplaces where there is a physical hazard in the work process in the form of a hot working climate. In a hot working climate, the body regulates its temperature by accelerating the evaporation of sweat by widening (vasodilation) of blood vessels which results in increased blood circulation and increased capillaries accompanied by physiological responses, such as increased pulse and blood pressure. This study aims to analyze the relationship between the work of heat and blood pressure of workers in the Laundry Installation of RSUP Dr. Sardjito Yogyakarta. This study uses quantitative research design using a cross sectional approach. The population in this study were Laundry Installation workers with a sample of 35 respondents taken with total sampling. Data analysis using Chi-Square. The results showed that there was a relationship between the hot work climate and workers’ blood pressure (p = 0.03) and there was a relationship between the hot working climate and diastolic blood pressure of workers in the Laundry in RSUP Installation. Sardjito Yogyakarta (p = 0.01).

Keywords: Hot Work Climate, Blood Pressure

Introduction
Hospitals as a means of improving health efforts that carry out health services, turned out to have positive and negative impacts on the surrounding environment. Hospitals in the implementation of inpatient care, emergency services, medical and non-medical services use technology that can affect the surrounding environment1. Hazard factors that exist in hospitals can cause workplace accidents or work-related illnesses, not only to the direct perpetrators who work in hospitals, but also to patients and visitors to hospitals who have the potential to experience the risk of workplace accidents and work-related illnesses2.

Dr. Hospital Sardjito is a Type A Hospital that is managed by the Ministry of Health which is a reference for the Special Province of Yogyakarta (DIY) and southern Central Java. Dr. Hospital Sardjito, there are 29 installations, each of which has the potential for physical, biological, chemical, psychological and ergonomic hazards which can cause work accidents or work-related illnesses, one of which is a Laundry Installation. Laundry Installation at Dr. RSUP Sardjito Yogyakarta has a risk of danger, one of which is a physical factor which is a hot working climate caused by the machines used during the work process, which take place from the washing process to drying, ironing, folding to being redistributed. Hot work climate is one of the physical factors that has the potential to cause harm and health problems when working in extreme hot and cold conditions with levels that exceed the Threshold Limit Value (NAV)3.

Hot work climate can affect health, one of them is increase in blood pressure. Blood pressure is a pressure produced by the blood from the circulatory system or the vascular system against blood vessel walls4. Based on the results of a preliminary survey conducted on September 13, 2017 at the Laundry Installation of RSUP Dr. Sardjito Yogyakarta, the result of measurement of hot work climate based on hospital data carried out on previous measurements was carried out 2 times, namely in December 2016 the ISBB results were 31°C and in June 2017 the ISBB was 36°C. The government has policies related to the workplace...
climate, which is about the Physical Factor Threshold Value and Chemical Factors in the Workplace, with the ISBB light category workload not exceeding the NAB of 31 °C. From the results of interviews with 7 workers from 35 work workers, grievances were felt at the place, often feeling excessive headaches, dizzy eyes, feeling tired, uncomfortable and sweating easily. Based on the description above, the researcher is interested in analyzing the relationship between the hot working climate and the blood pressure of workers at the Laundry Installation of RSUP Dr. Sardjito Yogyakarta.

**Research Method**

This type of research is a quantitative design, using cross sectional approach, where the independent variables (hot work climate) and dependent variables (blood pressure) are observed only once at the same time. This research was conducted on February 27, 2018, and was carried out at the Laundry Installation of Dr. RSUP Sardjito Yogyakarta. The number of workers in the Laundry Installation as many as 35 people with samples taken from the entire population of workers in the Laundry Installation. The variable hot working climate uses the appropriate category <31°C and does not match ≥ 31°C for the blood pressure dependent variable using the category of increase and decrease. The analysis used is univariate and bivariate analysis with the provisions of the test Chi-Square. If \( p \) value <0.05, then \( H_0 \) is rejected and if \( p \) value > 0.05, then \( H_0 \) is accepted by

**Finding**

**Characteristics of Respondents**

Based on the results of data collection of workers in the Laundry Installation, the respondent’s age is at least 19 years and maximum age is 57 years. And it was found that some age groups more than 80% experienced an increase in blood pressure systolic in the early adult group, late adult, early elderly and late elderly. And almost all age groups experience an increase in blood pressure diastolic by 100% for late adolescents, late adults and early elderly. Based on gender distribution in the Laundry Installation section, all male workers experience increased pressure, both systolic blood pressure and blood pressure diastolic by 100%. Meanwhile, some more than 70% of women also experience an increase in systolic blood pressure and blood pressure diastolic.

The history of blood pressure showed that the majority in the category did not have a history of blood pressure which many experienced an increase in systolic blood pressure and blood pressure diastolic by 87%. And in the category that has a history of blood pressure most also experienced an increase in systolic blood pressure and blood pressure diastolic by 75% and 100%. And more than 80% of workers who work with a light workload also experience an increase in systolic blood pressure and blood pressure diastolic by 85.8% and 88.6%.

**Hot Work Climate**

<table>
<thead>
<tr>
<th>Measurement Point Measuring</th>
<th>Results (C)</th>
<th>NAB (31 C)</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Point 1</td>
<td>25</td>
<td>According</td>
<td>33,51</td>
</tr>
<tr>
<td>Point 2</td>
<td>33</td>
<td>Not Appropriate</td>
<td></td>
</tr>
<tr>
<td>Point 3</td>
<td>35</td>
<td>Not Corresponding</td>
<td></td>
</tr>
</tbody>
</table>

In table 1, based on the measurement results of hot work climate carried out at 3 points in the Laundry Installation RSUP Dr. Sardjito obtained measurements at point I (administrative space) where the measurement results obtained were 25°C, point 2 (dirty area) the measurement results obtained were 33°C and at point III (clean area) the measurement results obtained were equal to 35°C. The results of the measurement of the hot working climate in the Laundry Installation section of Dr. RSUP Sardjito shows two points that ≥31°C these results exceed the NAB that has been set. 5.
Changes in Blood Pressure

Table 2. Changes in Blood Pressure after Exposure to Hot Work Climate at Respondents in the Laundry Installation Section of RSUP Dr. Sardjito

<table>
<thead>
<tr>
<th>Blood Pressure</th>
<th>Changes in Blood Pressure</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Increases</td>
<td>Decreased</td>
</tr>
<tr>
<td></td>
<td>Frequency</td>
<td>%</td>
</tr>
<tr>
<td>Systole</td>
<td>30</td>
<td>85.7</td>
</tr>
<tr>
<td>Diastole</td>
<td>29</td>
<td>82.9</td>
</tr>
</tbody>
</table>

Based on table 2. From the results of blood pressure measurements systolic showed that of the 35 respondents in the Laundry Installation there were 30 respondents (85.7%) experiencing an increase, while for blood pressure diastole there were 29 respondents (82.9%) experiencing an increase in the Relationship of Work Climate to increased blood pressure

Table 3. Work climate to increase systolic blood pressure

<table>
<thead>
<tr>
<th>Work</th>
<th>Climate Sistole Blood Pressure Change</th>
<th>Total</th>
<th>Sign</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Increases</td>
<td>Decrease</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>-----------</td>
<td>-----------</td>
<td>---</td>
</tr>
<tr>
<td>In Accordance with</td>
<td>2</td>
<td>50</td>
<td>2</td>
</tr>
<tr>
<td>Not Corresponding</td>
<td>28</td>
<td>90.3</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 4. Work climate towards increased blood pressure diastole

<table>
<thead>
<tr>
<th>Working Climate</th>
<th>Change of Diastole Blood Pressure</th>
<th>Total</th>
<th>Sign</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Increases</td>
<td>Decrease</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>-----------</td>
<td>-----------</td>
<td>---</td>
</tr>
<tr>
<td>Corresponding</td>
<td>2</td>
<td>50</td>
<td>2</td>
</tr>
<tr>
<td>Not Corresponding</td>
<td>29</td>
<td>93.5</td>
<td>2</td>
</tr>
</tbody>
</table>

Based on table 4. statistical test results Chi-Square show Sign for 0.03 < 0.05 which means that Ho is rejected so that there is a relationship between the hot work climate and blood pressure systolic and in table 5. Sign 0.01 < 0.05, which means that Ho is rejected so there is a relationship between the hot work climate and blood pressure diastolic. Workers who work in points II and III, namely workplaces that do not comply with the NAB, have experienced an increase in blood pressure, both systolic blood pressure and blood pressure diastolic.
Discussion

The measurement of hot working climate in the Laundry Installation section is carried out at 3 points, namely point I (administrative space) where there are 4 workers at that point, point II (dirty area) there are 6 workers, and point III (clean area) there are 25 the worker. Point I for employees of Laundry Installation staff, while point II and point III are places where workers do work, where workers also work close to machines. At the time of conducting research the measurement of the hot working climate that was felt when entering into the room was heat because the machines were adjacent to the activities of the workers and the rooms were not so wide. The results of the measurement of hot work climate obtained at point I is 25°C, at point II of 33°C and at point III of 35°C.

According to the Minister of Manpower and Transmigration Regulation based on the calculation of workload obtained is a light workload with hours working 8 hours, the hot working climate must not exceed the Threshold Limit Value (NAB) which is equal to ≥31°C. Based on these regulations the work climate is hot in the Laundry Installation section of Dr. RSUP Sardjito has 2 points that exceed the Threshold Value (NAB), which is point II (dirty area) and point III (clean area). At point I (administration room) in accordance with the Threshold Value (NAB) because the room is an air-conditioned room for employees of Laundry Installation staff.

Blood pressure measurements were carried out on 35 respondents, of which 35 respondents spread over 3 points, namely point I there were 4 workers, point II Based on the results of the Statistics test Chi-Square, a hot working climate with blood pressure systolic workers at the Laundry Installation RSUP Dr. Sardjito obtained a significance value of 0.03 (P <0.05) so that Ho was rejected, which means there is a relationship between the hot work climate and blood pressure systolic in workers at the Laundry Installation of RSUP Dr. Sardjito. This research is in line with the research conducted by Jaswin (2004), which shows that there is a relationship between heat pressure and blood pressure systolic in the production workforce of PT. Tjokro Bersaudara with a sig value of 0.03 <0.05 and there is a relationship between heat pressure and blood pressure diastolic in the production workforce of PT. Tjokro Bersaudara with a sig value of 0.04 <0.05. This shows that there is a relationship between the hot working climate and the blood pressure of workers in the Laundry Installation section of Dr. RSUP Sardjito Yogyakarta and point III there are 31 workers, where point I and point II have a hot work climate that exceeds NAB. In addition, the results of the measurements also showed that workers who worked in that place experienced a lot of increased blood pressure both systolic blood pressure and blood pressure diastolic which was equal to 90.3% and 93.5%. Meanwhile, those who experienced a decrease in systolic blood pressure and blood pressure diastolic only 9.7% and 6.4%. Furthermore, at point I workplaces that have a work climate in accordance with the NAB there are 4 workers, of which 2 (50%) workers have increased systolic blood pressure and blood pressure diastolic due to other factors, one of which is a history of blood pressure. Whereas, who experienced a decrease in systolic blood pressure or blood pressure diastolic there were 2 (50%) respondents.

Conclusion

Measurement of hot working climate in the Laundry Installation section of Dr. RSUP Sardjito is done at 3 points, namely point I (administrative space) 25°C, point II (dirty area) 33°C, and point III (clean area) 35°C, where the highest measurement results at point II and point III value >31°C exceeds the Threshold Value (NAB). Blood pressure measurement in the respondent of the Laundry Installation of RSUP Dr. Sardjito majority experienced an increase in systolic blood pressure and blood pressure diastolic by 90.3% and 93.5%. The statistical test results obtained the relationship between hot work climate and blood pressure systolic in workers in the Laundry Installation obtained a significance value of 0.03 (P <0.05) and the relationship between hot work climate with blood pressure diastolic in workers on the Laundry Installation and obtained a significance value of 0.01 (P <0.05).
**Conflicts of Interest:** all authors have no conflict of interest to declare.

**Source of Funding:** the source of research cost from self.

**Ethical Clearance:** the study was approved by the Surakarta University’s Ethical Bord of the Muhammadiyah, Faculty of Health Sciences, Public Health, Study Program.

All subjects have been fully informed about the procedure for signing an informed consent form.

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2. RI Ministry of Health. Occupational Safety and Health Guidelines at Hospital Pharmacy Installation. Jakarta Indonesia; 2006
Analysis of Worker’s Level of Knowledge on Handling Chemicals in Oil and Gas Industry Laboratory of Pt “X” Indonesia

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Abstract

The oil and gas industry is one of a large-scale industry, which is when carrying out the production process, they contain several hazard posed that can threaten labor, assets, and other people in industrial environment such as chemical hazards. The chemical groups have special characteristics in disturbing and threatening labor. Knowledge is one of the important factors in preventing work accidents and work-related diseases due to chemicals. This study is a descriptive study, and the data was collected by observational. The object of research in this study is knowledge of workers on handling chemicals. The sample is a total population of 28 workers. The results of the research obtained were that most workers already had adequate knowledge regarding handling chemicals in the laboratory. The highest concentration of chemicals in the laboratory is Benzene (19.1 ppm), Toluene (30.56 ppm) and Xylene (20.48 ppm). The conclusion of this study is that knowledge is a very important domain for the formation of a person’s behavior. In this case, it is related to the handling of chemicals found in the laboratory. If the level of knowledge of the workers is high, the handling of chemicals in the workplace can be carried out maximally, so that workplace accidents can be prevented and the safety of workers can be maintained.

Keywords: chemicals, workers’ knowledge, laboratory.

Introduction

OHS (Occupational Health and Safety) is a program created by workers and employers as an effort to prevent accidents and occupational diseases (OD). Based on Article 86 paragraph (1) letter a of Law Number 13 of 2003 concerning Labor, every worker has the right to obtain protection for Occupational Health and Safety. The lack of care of the company towards OHS is reflected in the high rate of work accidents and the less optimal detection of OD¹.

The International Labor Organization (ILO) stated that 160 workers experienced work-related illness every 15 seconds². Workplace accidents and OD are a health and economic burden in Indonesia because not only do they need services and health costs, but they also reduce the productivity. Occupational Disease is a disease caused by work or work environment that will result in partial or total disability. Part of the defect is the loss or non-functioning of some members of the body of labor forever. Whereas total disability is a state of labor without being able to work at all forever³.

In general, the causes of OD can be grouped into five groups, namely the physical group: noise, radiation, temperature, very high pressure, vibration, poor lighting. Chemical group: chemicals used in work processes, as well as those found in the work environment, can be in the form of dust, steam, gas, solution, clouds or fog. Biological group: bacteria, viruses or fungi. Physiological group: usually caused by workplace arrangement and work methods. Psycho-social group: stressful work environment.

The chemical group has special characteristics in disturbing and threatening the safety and health of...
workers. This can happen because some chemicals have physical and chemical properties like invisible color, odor that does not smell. Therefore handling and knowledge of chemicals in the workplace is very important. Knowledge is one of the important factors in preventing work accidents and work-related diseases due to chemicals. Through good knowledge, the workforce can take the right actions at work.

The oil and gas industry is a large-scale industry with labor intensive and capital intensive. In carrying out the production process, there are several dangers posed that can threaten human. Some of these hazards are noise due to work processes, extreme temperatures, and lighting in the workplace. Chemical hazards such as benzene, toluene, xylene and H₂S. Biological hazards such as the presence of wild animals, bacterial, viruses or fungus contamination. Before being marketed, all the oil and gas that has been produced will be tested in the laboratory. This shows that workers in the laboratory are also exposed to chemicals hazards.

**Material and Method**

This study is a descriptive study, with observational to collecting some data. Based on the method of retrieval of data, this research is observational, because the data obtained through observation and not being treated on the object of research.

The object of research in this study is the workers’ knowledge on handling chemicals. The population was 28 workers in the oil and gas industry laboratory. The sample is a total population of 28 workers.

The data collected is primary data and secondary data. Primary data obtained from interviews are workers ‘s knowledge regarding chemicals. This data includes the workers ‘s knowledge on the identification of chemicals, how to handle products, regarding the use of RPE, regarding maintenance of Respiratory Protective Equipment (RPE) and regarding management support. Secondary data is obtained from data recorded in the oil and gas industry, such as: industrial profile data, and data on the number of workers.

**Findings**

Data collection was conducted on 28 respondents who worked in oil and gas industry laboratory of PT “X” Indonesia by distributing questionnaires and conducting interviews directly.

A. Overview of Air Chemical Concentration in the PT “X” Indonesia Oil and Gas Laboratory

Based on the chart above, it can be seen that the highest concentration of chemicals in oil and gas laboratory of PT “X” Indonesia in the form of Benzene is 19.1 ppm, the highest concentration of Toluene is 30.56 ppm, and the concentration of Xylene is 20.84 ppm, while the concentration of NH₃, H₂S, CO and H₂SO₄ in the laboratory is still not detected which is 0.00. Although the concentration of these chemicals has not exceeded the TLV (Threshold Limit Value) that has been determined based on the Minister of Manpower and Transmigration Regulation of the Republic of Indonesia Number PER.5 / MEN / X / 2018 concerning occupational health and safety and work environment, it still needs to be watched out for the effect the exposure caused can affect the health and safety of workers.

B. Questionnaire Result of Oil and Gas Laboratory of PT “X” Indonesia

1. Workers’ Knowledge Level on Identifying Chemical Products

   Based on the results of the questionnaire, it was found that 100% of workers knew the identification of chemical products containing hazardous materials in their workplaces (BTX, caustic soda, DMDS), and 100% of workers knew the source of information regarding chemical products in the workplace.

2. Level of Worker Knowledge on How to Handle Products

   Based on the results of the questionnaire 71% of workers stated that there were measurements of gas testing in their workplaces. 100% of workers know the type of respiratory protective equipment that must be used in handling the hazard products they encounter at work.
3. Workers’ Knowledge Level regarding the Use of RPE

Based on the results of the questionnaires, 100% of workers know the health effects of chemical products in their workplaces, workers also know the type of respiratory protective equipment they use, know how to use the correct RPE, know the disposable RPE, know that disposable RPE can only be used once, knowing that reusable RPE cannot be used alternately, knowing the function of the RPE they use, and 43% of workers stating that there are no complaints when using RPE.

4. Maintenance of Respiratory Protective Equipment (RPE)

Based on the results of the questionnaire, it was found that 79% of workers stated that there was an RPE maintenance procedure at their place of work. 68% stated that treatment performed on RPE can affect the effectiveness of RPE function. 71% of workers stated that they knew the condition of the RPE that needed cleaning. 96% of workers stated that they kept RPE in clean and sealed plastic before putting it in a locker. 79% of workers stated that they had checked the RPE condition before using it. 79% of workers stated that they knew how the RPE could be used.

5. Level of Knowledge regarding Management Support

Based on the results of the questionnaire, it was found that 100% of workers had received RPE-related training, 100% of workers stated that the RPE they received was in accordance with their type of work, and 100% of workers stated that there had never been cases of shortness of workers when wearing RPE.

Chemicals in the Laboratory

Benzene, Toluene and Xylene or commonly known as BTX are several types of air pollutants which are Polycyclic Aromatic Hydrocarbon (PAH) compounds. PAHs are formed due to incomplete combustion of organic matter, spread to the environment and in mixed form. The main sources of exposure of PAH to humans come from the work environment, passive and active smokers, food and water and air pollution\(^4\).

BTX is a chemical including chemicals that are toxic to health, both carcinogenic and trigger cancer and increase oxidative and non-carcinogenic stresses such as affecting the hematopoietic system, central nervous system and reproductive system\(^5\).

1. Benzene

Exposure to benzene on humans through inhalation is carcinogenic. The presence of benzene exposure in the work environment has been associated with an increased incidence of acute and chronic myeloid myeloblastic or erythroblastic leukemia and lymphoid leukemia in workers.

The Indonesian National Standard 2005 which refers to Minister of Manpower Regulation No. 13 of 2011 contains the Threshold Value (TLV) of the time weighted average of chemicals in the workplace air, with the number of hours worked 8 hours per day or 40 hours per week, stating that benzene included in group A2 (chemicals that are estimated to be carcinogens for humans) have NAB of 10 ppm or 32 mg/m\(^3\) of benzene in the air\(^6\).

In contrast to xylene and toluene, benzene is a material that is proven to be carcinogenic which can interact with RNA, proteins or other molecular compounds that can trigger carcinogenic effects without being directly related to exposure concentration\(^7\).

The health effects of benzene exposure at low levels can cause dizziness or drowsiness, a rapid heartbeat, headaches, tremors, and confusion. At a higher level can cause unconsciousness or even death. Long-term exposure can have serious health consequences, especially in the bone marrow, or through loss of red blood cells, which can cause anemia. This can disrupt the immune system, and make patients vulnerable to other diseases, benzene can also cause cancer, especially leukemia and other cancers in the blood.

2. Toluene

Toluene is one of the aromatic hydrocarbon compounds, colorless substances, flammable liquid with a distinctive aroma, not corrosive, explosive vapor, insoluble in water but soluble in ketones, alcohols, esters and other aromatic hydrocarbon compounds\(^8\).
### Effect of toluene toxin:

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 ppm</td>
<td>Threshold of pungent odor</td>
</tr>
<tr>
<td>37 ppm</td>
<td>Still acceptable to humans</td>
</tr>
<tr>
<td>50-100 ppm</td>
<td>Subjective complaints (fatigue, feeling sleepy and headache)</td>
</tr>
<tr>
<td>200 ppm</td>
<td>Eye and respiratory tract irritation, cognitive damage, headache, dizziness, hangover, fatigue, confusion, insomnia</td>
</tr>
<tr>
<td>300 ppm</td>
<td>Coordination damage if exposure is up to 8 hours</td>
</tr>
<tr>
<td>400 ppm</td>
<td>Eye, respiratory, and tear gland irritations, skin paresthesia, signs of lack of coordination and mental disorders if severe for more than 8 hours</td>
</tr>
<tr>
<td>500-600 ppm</td>
<td>Anorexia, staggering pathways, weak nausea, reduced memory, reduced time to recreation</td>
</tr>
<tr>
<td>800 ppm</td>
<td>Very nauseous (after 3 hours of exposure), confused, low self-control, very anxious, memory loss (insomnia) for several days</td>
</tr>
<tr>
<td>1500 ppm</td>
<td>Uncoordinated, very tired</td>
</tr>
<tr>
<td>4000 ppm</td>
<td>Can cause damage to response, necrosis and death</td>
</tr>
<tr>
<td>10000-30000</td>
<td>Necrosis, death.</td>
</tr>
</tbody>
</table>

### Xylene

Xylene is a colorless, flammable, volatile and sweet-scented liquid\(^9\). Xylene is naturally found in kerosene, coal and forest fire processes or through the process of automation of petroleum hydrocarbons. On an industrial scale, xylene is produced through the heating process of organic compounds and catalyst processes for kerosene products\(^10\).

Xylene exposure can also occur through inhalation (breathing), ingestion, eye contact and in some rare cases, xylene can be absorbed in small amounts on the skin, which is the main effect that can arise from xylene exposure is a result of breathing in xylene vapor\(^10\).

The negative impact that can be caused from xylene exposure is depression in the central nervous system, with symptoms such as headache, dizziness, nausea and vomiting. Like benzene, xylene can also cause a decrease in red blood cells (anemia)\(^11\).

Xylene which is ingested into the body can cause stomach problems and cause toxic effects on the liver. Exposure to high concentrations of xylene vapors that occur acutely can cause impaired function and swelling and bleeding\(^11\).

Xylene concentrations below 200 ppm will irritate the eyes and membranes of the lenses, while at high concentrations xylene can cause narcotic effects. Estimated LD50 orally in humans is 50 mg / kg\(^12\).

### The Importance of Knowledge

Knowledge is the result of knowing, and this happens to someone who does sensing a particular object. Most human knowledge is obtained through the eyes and ears. Knowledge is a very important domain for the formation of one’s behavior. Besides knowledge or cognitive is a domain that is very important for the formation of one’s actions (Overt Behavior).
Some studies that have been conducted also show that a conclusion is obtained that there is a relationship between knowledge and attitude with the use of personal protective equipment\textsuperscript{13}.

Knowledge of the characteristics of chemicals in the workplace can provide facts and information related to handling the chemicals and take actions when the problems occur. One important knowledge must be possessed while in the laboratory and direct exposure to chemicals including: Knowledge of the identification of chemicals, Knowledge of how to handle products, Knowledge of how to use and maintain RPE, and Knowledge of Management Support.

Hazard identification is an effort to prevent and reduce the occurrence of workplace accidents that occur in the company, and avoid and minimize risks in the right way by avoiding and reducing the risk of workplace accidents and their control in carrying out repair and maintenance activities so that the process is safe. Hazard identification includes identification of aspects of the Company’s environmental impacts on the environment and surrounding residents in the Company’s area\textsuperscript{14}.

Knowledge of how to use and maintain respiratory protective equipment (RPE) is very important because it is a device that can protect workers from exposure to hazardous substances. The use of respiratory protectors prevents workers from directly inhaling contaminants in the work area.

Respiratory Protective Equipment according to Regulation of the Minister of Labour and Transmigration Number 8; 2010 concerning Personal Protective Equipment is a protective device that serves to protect respiratory organs by channeling clean and healthy air and / or filtering out contamination of chemicals, micro-organisms, particles in the form of: dust, fog (aerosol), steam, smoke, gas / fume and so on\textsuperscript{15}.

Based on its function, RPE is divided into: Respirator which functions to purify the air (air purifying respirator), respirator which functions to supply oxygen or air (water supplying respirator), respirator with a canister containing chemicals, mechanical respirator (mechanical respirator), filter combination respirator and chemicals, respirators with suppliers of air or oxygen.

**Conclusion**

From the research that has been done, some conclusions can be drawn as follows.

1. Most workers already have adequate knowledge regarding handling chemicals in the oil and gas laboratory of PT “X” Indonesia in accordance with procedures that have been included in the OHS norms in the workplace.

2. The highest concentration of chemicals in the oil and gas industry laboratory of PT “X” Indonesia is Benzene (19.1 ppm), Toluene (30.56 ppm) and Xylene (20.48 ppm).

3. Knowledge is a very important domain for the formation of a person’s behavior, in this case is related to the handling of chemicals found in the oil and gas industry laboratory of PT “X” Indonesia.

4. If the level of knowledge of the workers is good, then the handling of chemicals in the workplace can be done to the maximum, so that work accidents can be prevented and the safety of workers can be maintained.

**Ethical Clearence:** This Research Already pass Ethical Clearence From FPH Universitas Airlangga

**Conflicts of Interest:** The authors have no conflicts of interest to declare for this study.

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Climate Relationship Work with Fatigue Employees Working in Laundry Plant Dr. Sardjito

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Abstract
Laundry Installation is one of 29 installations in Hospital Dr. Sardjito. Laundry installation has 35 employees who have worked with exposure to high temperatures up to 35°C. The hot working climate be the cause of fatigue on employees. The purpose of this study was to Determine the relationship between the work climate in employees with work fatigue Hospital Dr. Laundry Installation Sardjito Yogyakata. The study design used is cross sectional. The population in this study as many as 35 people. The sampling technique used is total sampling the entire population of employees that numbered 35 people. The results of the study with the statistical tests Man Whitney p value 0.022 Obtained value <0.05 the which means that Ho refused so that there is work climate relationship with work fatigue of employees working in the department of Dr. Laundry Installation Sardjito. The advice given to the installation related to that with the addition of appropriate ventilation in the standard installation space doing laundry and job rotation system working parts so that employees do not feel monotonous with the work performed.

Keywords: Climate Work, work fatigue, workers of Installation Laundry

Introduction
The incidence of cases of occupational diseases continues to increase each year, one of occupational diseases in the hospital. The hospital has a variety of installations that have high hazard potential, ranging from the installation of the laundry, pharmacy, nutrition installation, forensic medicine laboratory, steam boilers, and warehouse B3. Laundry installations cited as one part of a hospital where a high risk that this installation has a temperate working environment hot work. Working climate itself becomes part of the physical factors that potentially dangerous health problems both hot and cold taupon with levels that exceed the Threshold Limit Value (TLV)¹. Work climate that meets the health requirements will affect the optimal working efficiency².

General Hospital Dr. Sardjito a regional referral hospital for the province of Yogyakarta (DIY) as well as the southern part of Central Java. Hospital Dr. Sardjito has 29 installations, which of the various installations that have a high hazard potential, one Installation Laundry. Installation laundry (Laudry) consists of three parts, namely the work area clean room area, dirty room area, and the administrative staff room. Dirty room clean room area and the area carry out activities ranging from separation, washing, drying, folding and ironing linen up until ready to be distributed back. While the administrative staff conducting space-related administrative office Laundry Installation.

Based on the preliminary survey performed at the laundry, all the employees working in the work environment with a hot working environment that does not meet (NAB). Based on data from the Hospital of climate measurement results has been done before as many as 2 times the last measurement in December 2016 obtained results Wet and Ball Temperature Index (WBGT) equal to 31C and in June 2017 WBGT result of 36C. When compared with the standard working
climate in Indonesia established by Permenakertrans No. Per.13 / MEN / X / 2011 Threshold Limit Values for the working climate that is 31°C with a light workload, the results exceeded the NAB³.

Based on initial observations showed that the researchers feel the heat directly working climate that is in Installation Laundry. It was marked with only a few minutes of being in the room already felt hot and hot. As for the initial interview conducted on 10 employees all complained of feeling tired, weak, and thirst. Results of research conducted Sari⁴ concluded that there is significant influence among the working climate to dehydration and heat exhaustion in labor work in the boiler section Albasia Sejahtera Mandiri PT. The results of another study that is conducted by⁵ concludes that there are climatic influences hot work against fatigue in metal smelting section Batur Jaya Ceper cooperative, Klaten. The purpose of this study was to determine the relationship of the working climate with job burnout in employees cleaning installations RSU Dr. Sardjito

**Research Method**

This type of research used in this research is quantitative research design with observational and cross-sectional approach. The population in this study were all employees of the department Dr. Laundry Installation Sardjito totaling 35 people. The number of samples in this study were 35 taken with total sampling sampling techniques.

The independent variable in this study is the work climate, the dependent variable in this study were fatigue, and variable spam is measured in this study were age, gender, and workload while confounding variables that are not measured in this study is the noise, vibration and length of employment. Measurement of working climate with Heat Stress Monitor brands Area Quest 10 and Fatigue Temp work using a questionnaire fatigue. Implementation of measurements were carried out at 09.30 am.

**Findings**

1. **characteristics of Respondents**

Table 1. Frequency and Percentage Distribution Characteristics of respondents as well as the rate of job burnout Laundry Installation Hospital Emp

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Frequency</th>
<th>Work fatigue</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Low</td>
<td>moderate</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>teens End</td>
<td>3</td>
<td>8.6</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Early adulthood</td>
<td>7</td>
<td>20.0</td>
<td>5</td>
<td>71.4</td>
<td>1</td>
</tr>
<tr>
<td>adults final</td>
<td>8</td>
<td>22.9</td>
<td>2</td>
<td>25</td>
<td>6</td>
</tr>
<tr>
<td>Early elderly</td>
<td>11</td>
<td>31.4</td>
<td>4</td>
<td>36.3</td>
<td>5</td>
</tr>
<tr>
<td>elderly End</td>
<td>6</td>
<td>17.1</td>
<td>2</td>
<td>33.3</td>
<td>2</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Man</td>
<td>18</td>
<td>51.4</td>
<td>6</td>
<td>33.3</td>
<td>11</td>
</tr>
<tr>
<td>woman</td>
<td>17</td>
<td>48.6</td>
<td>7</td>
<td>41.1</td>
<td>6</td>
</tr>
<tr>
<td>Workload</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Workload</td>
<td>35</td>
<td>100</td>
<td>13</td>
<td>37.1</td>
<td>17</td>
</tr>
</tbody>
</table>
Based on Table 1. The frequency distribution and percentage of employees Installation unknown Laundry most banyaj employee age is 46-55 years older initial total of 11 people (31.4%). As for the age is at least 17-25 years late teens totaling 3 (8.6%). While the frequency distribution and percentage of employees Installation gender Laundry Hospital Dr. Sardjito are males were 18 people (51.4%) and women were 17 people (48.6%). The frequency distribution and percentage of employees' workload Laundry Installing Hospital Dr. Sardjito is a light workload, as many as 35 people or as much as 100% of employees received workload during work including light workload.

Then the measurement results with fatigue life of employees working at Hospital Laundry Installation Dr. Sardjito is known that at the end of the adolescent age category all employees experience job burnout Low totaling 3 (100%), for the end of the adult age categories experienced job burnout mostly lower, amounting to 5 people (71.4%), adult age categories end of most experienced job burnout were amounting to 6 people (75%), older age categories beginning mostly experienced job burnout were amounting to 5 people (45.4%) and for the age category final elderly employees who experienced job burnout low 2 (33.3%), fatigue was 2 JV (33.3%) and high job burnout 2 (33.2%).

The measurement results sex with job burnout of employees known that male employees mostly experienced job burnout were a total of 11 people (61.1%), whereas women employees working mostly mild fatigue, amounting to 7 people (41.1%). Based on the measurement results of the workload with employee fatigue LaundryInstalling Hospital Dr. Sardjito known to all employees, amounting to 35 people suffered light workload, with a light workload of employees who experienced job burnout lower categories numbered 13 persons (37.1%), fatigue moderate category amounted to 17 (48.6%) and fatigue higher category of work of 5 people (14.3%).

2. Work climate

Table 2. Results of the Climate Measurement Work on the installation of Dr Sardjito Hospital Laundry

<table>
<thead>
<tr>
<th>Measuring point</th>
<th>WBGT</th>
<th>NAB</th>
<th>mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>°C</td>
<td>³1C</td>
<td></td>
</tr>
<tr>
<td>Point one (dirty room area)</td>
<td>33</td>
<td>≥NAB</td>
<td></td>
</tr>
<tr>
<td>Colon (clean room area)</td>
<td>35</td>
<td>≥NAB</td>
<td>33.51</td>
</tr>
<tr>
<td>Point three (space administration staff)</td>
<td>25</td>
<td>&lt;NAB</td>
<td></td>
</tr>
</tbody>
</table>

Based on the data in Table 2 WBGT measurement results Installation work climate at Dr. Hospital Laundry Sardjito indoor WBGT value is point one dirty room area ie ≥ 33° 31c, colon clean room area which is 35°C ≥ 31C, and point three administrative staff room is 25°C <31C.

3. Work fatigue

Table 3. Results of Measurement fatigue on the part of the Installation Work of Dr. Sardjito Hospital Laundry

<table>
<thead>
<tr>
<th>Fatigue category of work</th>
<th>Frequency</th>
<th>Presentation (%)</th>
<th>mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (0-21)</td>
<td>13</td>
<td>37.1</td>
<td></td>
</tr>
<tr>
<td>Medium (22-44)</td>
<td>17</td>
<td>48.6</td>
<td>25.03</td>
</tr>
<tr>
<td>High (45-67)</td>
<td>5</td>
<td>14.3</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>
Based on the data in Table 3 the results of measurements of employee fatigue Laundry Installing Hospital Dr. Sardjito that employees who experience fatigue with low fatigue category amounted to 13 employees (37.1%), fatigue was numbered 17 employees (48.6%), high work fatigue, amounting to 5 employees (14.3%).

4. Climate relations job with job burnout

Table 4. Results of analysis of the relationship between the working climate with fatigue Work on the installation of Dr Sardjito Hospital Laundry

<table>
<thead>
<tr>
<th>Work climate</th>
<th>Low</th>
<th>moderate</th>
<th>High</th>
<th>Total</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;NAB</td>
<td>4</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>≥NAB</td>
<td>9</td>
<td>29.03</td>
<td>17</td>
<td>54.83</td>
<td>5</td>
</tr>
</tbody>
</table>

Based on the results of climate measurements known to work with job burnout at work climate <NAB all employees of the department Dr. Laundry Installation Sardjito experiencing job burnout light category as many as 4 people (100%), while the working climate ≥NAB most employees experienced job burnout were as many as 17 people (29.03%). As for the Man Whitney statistical test showed p value of 0.012 <0.05, which means that Ho refused.

Discussion

Based on the results of climate measurements of work done in the three measurement points which point one in the dirty room area, two points in the clean room area and a point in the space of three administrative staff is known that the measurement results indicate there are two categories of work climate that is <NAB and ≥NAB. To point one and point two results are ≥NAB 31C and three point <NAB 31c. According to the regulations of the Minister of Manpower and Transmigration No. PER.13 / MEN / X / 2011 on the threshold values for the physical factors of climate hot working with the type of work that has a workload of light category which worked continuously (8 hours per day) with working time regulations (75%) and time off (25%) should not exceed 31C WBGT. Based on these regulations Installation work climate at Dr. Hospital Laundry Sardjito exceeding the threshold value set so high risk for employees. The measurement results on employee job burnout Laundry Installation Hospital Dr. Sardjito shows that the average value of fatigue is 25.03 categorized as moderate job burnout, as many as 13 people experiencing job burnout lower category (37.1%), as many as 17 people (48.6%) experiencing job burnout medium category, and as many as five people (14.3%) experienced fatigue high category. Based on the results of these measurements in the highest percentage of job burnout category being. Fatigue experienced by employees of the department Dr. Laundry Installation Sardjito still a complaint because it is still a category fatigue was not until the decline in work performance characterized by weakening physical condition of workers The measurement results working climate with fatigue at the point of the room dirty area and a colon in the clean area where there are 31 employees who worked as 6 people in the room dirty area and 25 in the clean area known to result > NAB. In addition the measurement results also showed that employees who work in these places experiencing fatigue. For low work fatigue were 9 people (29.03%), job burnout were as many as 17 people (54.83%), and high work fatigue as much as 5 people (16.12%).

Based on the results of Man Whitney statistical test between the hot working environment with job burnout in employees cleaning installation Hospital Dr. Sardjito obtained p value 0.012 <0.05, then Ho is rejected means that there is a relationship between the working climate
with job burnout in employees Laundry Installation Hospital Dr. Sardjito. This study is in line with research Sari (2014), that there is influence between the hot working climate with dehydration and fatigue on the part of labor boiler with p value = 0.023 and p value = 0.000 which means ≤ 0.05. However, these studies are not consistent with research conducted Ristiyanto because the test results obtained statistical p value 0.111> 0, 05 then the test results stated insignificant or no employment relationship hot climate with job burnout in the boiler unit labor PT. Tiga Pilar Sejahtera Food Tbk Village Sepat, Masaran, Sragen, because due to the factors that affect the statistical test that health status, age and acclimatization.

The results of the research work climate with job burnout in employees Laundry Installation Hospital Dr. Sardjito is no relationship between the work climate at work fatigue as evidenced by the observation of a work environment where employees do laundry Installation work exposed to heat, where the heat source is derived from a washing machine, a dryer, and ironing machine. Although the condition of the room dirty area and a clean room area is equipped with a fan and exhauster which is quite a lot, which exhauster can suck the hot air from inside the room and throw it out at the same time sucking fresh air from outside into the room. However, lack of ventilation also be one of the causes of climate gets hot.

**Conclusion**

According to the research performed at the Hospital Dr. Laundry Sardjito daapat concluded that the measurement of WBGT at three measurement points which point one in the lounge area dirty 33c, colons in the clean room area of 35˚C, and the point in the space of three administrative staff 25˚C while the measurement results obtained job burnout employees who experience fatigue with mild fatigue criteria categories numbered 13 employees (37.1%), fatigue was amounted to some 17 employees (48.6%), and high fatigue amounted to 5 employees (14.3%). In addition, by Man Whitney statistical test between the working climate with job burnout show 0,012 p value <0.05, which means that there is a relationship between the working climate with job burnout in employees Laundry Installation Hospital Dr. Sardjito.

**Conflicts of Interest:** all authors have no conflict interest to declare.

**Source of Funding:** The source of this research costs from the self.

**Ethical Clearance:** The study was approved by the institutional Ethical Board of the Public Health, Airlangga University.

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Association of Noise Exposure and Physical Workload on Systolic Blood Pressure of Ceramic Industry Workers

Erike Anisa Nurshafa¹, Noeroel Widajati¹
¹Department of Occupational Health and Safety, Faculty of Public Health, Universitas Airlangga, Surabaya, East Java, Indonesia

Abstract

Noise is a major problem in modern industrial activities. The use of machines in industry and inseparable design of work between humans and machines makes workers exposed to noise as one of the physical hazards in the work environment. Noise can cause physiological reactions such as systolic and diastolic blood pressure disorders. However, systolic blood pressure response is more sensitive to changes. The systolic pressure after loading is normally up (20 mmHg), the rest is considered abnormal and is considered one of the risk factors for cardiovascular disease. Blood pressure is a multifactorial health indicator. In addition to noise, blood pressure is also influenced by the physical workload that workers receive as one of the factors of workload. The purpose of this study is to examine the relationship of noise and physical workload to an increase in systolic blood pressure. This is a cross sectional design and observational analytical study with statistical tests using spearman. Respondents were determined through simple random sampling technique that results in a sample of 36 ceramics industry workers in the production line section. The results showed the value of the noise relationship (r = 0.581) towards the increase in systolic blood pressure. Physical workload showed a relationship value of (r = 0.666) towards the increase in systolic blood pressure. The direction of the relationship between the independent variable and the dependent variable is positive, which means that the higher the noise level and physical workload, the higher the risk of abnormal systolic blood pressure.

Keywords: noise, physical workload, systolic pressure

Introduction

The main problem of occupational health in industrialized countries is noise. According to WHO in 1995 it was estimated that 14% of the workforce in the industrial country was exposed to noise of more than 90 dB. At least 7 million people or 35% of the total population in America and Europe were exposed to noisy 85 dB or more. In Poland it was estimated that 600,000 of the 5 million industrial workers had the risk of being exposed to noise. This illustrates the noise in the work environment has been a major problem that threatens the health of workers(1).

Noise is all the unwanted noise that comes from the tools of the production process or work tools which at a certain level can cause hearing loss. Noise is one of the important aspects in industrial hygiene and noise exposure is a form of hazard that can be controlled so as to reduce the impact caused. Noise exposure caused 54.7% of the workforce to experience non-auditory problems and only 18.7% of the workforce experienced auditory problems. Based on the study noise exposure has a greater non-auditory risk than that of auditory(2). Non-auditory effects due to noise in workers can be physiological, psychological and communication disorders. One of the non-auditory effects of noise exposure is a disturbance in cardiovascular and circulatory system caused by excessive production of adrenaline hormone, resulting in an increase in the frequency of heart rate and blood pressure(3). The production machine produces a noise level above the set threshold value of 85 dB. This noise is produced from production machines such as the GF
Box Feeder for the extruder stage which is used to check mass density so that the product is not easily broken and the crusher machine used to destroy ceramics that fails to pass the quality control stage.

Increased blood pressure is one factor in the occurrence of cardiovascular disease. According to WHO data, 31% of the 56.6 million deaths worldwide were caused by cardiovascular disease in 2012. In Indonesia cardiovascular disease recorded in Indonesian Basic Health Research was the highest cause of death by 1.5%\(^{(4)}\). Factors that affect blood pressure increase in workers are multifactorial. In addition to the danger that comes from the work environment in the form of noise, physical workload is also a factor that influences the risk of increased blood pressure. The physical workload can be in the form of lifting, running and carrying activities.

Physical work produces energy that meets energy consumption needs. Assessment of physical workload can be measured by an objective method of measuring the pulse during work. The higher the pulse count measured the higher the physical workload received by the worker. Pulse is one system that runs synergistically with blood pressure. Thus, the higher the burden of physical works, the higher the increase in blood pressure\(^{(5)}\). In accordance with the results of preliminary observations made by the researchers, the works in the ceramics industry is highly dependent on physical activities. Activities in the ceramics production process includes elevating the arrangement of ceramics to be assessed by quality control and preceded with packing process to ensure all ceramic surfaces get the perfect glazing process and arranging the ceramics on the pallet to be stored in the warehouse.

Based on the explanation, this study aims to analyse the relationship of work environment factors in the form of noise exposure and occupational factors in the form of physical workload to an increase in systolic blood pressure to workers in the ceramic production line.

Material and Method

This study aims to analyze the relationship between noise exposure and physical workload to increase systolic blood pressure in ceramic industry workers in the production line section. This is an observational analytic study because the researcher did not provide an intervention and only looked for the relationship of the variables studied. In terms of time, this is a cross sectional study because observations were made at one time. The study period was conducted in May 2019. The study population was 40 people and the sample was taken using the simple random sampling method with the Slovin formula which resulted in 36 research samples.

The independent variables tested were noise exposure and physical workload, while the dependent variable was systolic blood pressure. All variables were obtained through measurements as explained below.

1. Noise Measurement

The measurement of noise intensity was carried out at three points of the line production area of forming, glazing and packing & loading. Measurements were made using the Sound Level Meter (SLM) of Svantek type and carried out by the operator of the Occupational Safety Technical Unit of the East Java Province Manpower and Transmigration Office. Test method is based on SNI 7231: 2009 concerning Noise.

2. Measurement of Physical Workload

The measurement of physical workload uses the 10 pulse method to determine the Work Pulse Rate which is then classified based on the Cardiovascular Load formula (% CVL). Measurement was made using palpation method on the worker’s pulse by medical personnel of nurse. The measurement technique begins by pressing the on button on the stopwatch along with the first pulse and turning off the stopwatch on the 10th pulse. The number of seconds produced was then recorded. The pulse measurement was carried out four times by 8:30 a.m., 9:30 a.m., 10:30 a.m. and 11:30 p.m. to find out the average pulse rate at work.

\[
%CVL = \frac{100 \times (\text{Working pulse} - \text{Resting pulse})}{\text{Max pulse} - \text{Resting pulse}}
\]
Table 1. Categories of Physical Workload

<table>
<thead>
<tr>
<th>Workload Rate</th>
<th>CVL Category</th>
<th>CVL Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Light</td>
<td>&lt; 30 %</td>
</tr>
<tr>
<td>1</td>
<td>Moderate</td>
<td>30 to 60 %</td>
</tr>
<tr>
<td>2</td>
<td>Heavier</td>
<td>60 to 80 %</td>
</tr>
<tr>
<td>3</td>
<td>Heavy</td>
<td>80 to 100 %</td>
</tr>
<tr>
<td>4</td>
<td>Very heavy</td>
<td>&gt; 100 %</td>
</tr>
</tbody>
</table>

Source: Tarwaka, 2015

3. Measurement of Systolic Blood Pressure

Blood pressure measurement was carried out by medical staff of a nurse prior to working at 07:45 WIB and after working at 12:00 WIB. Measurements were performed using a manual sphygmomanometer and stethoscope.

Data collected from research results are processed in several stages. First, the data was coded numerically to maintain data privacy and facilitate data input activities. The data analysis activity aims to find the significance of the relationship, the strength of the relationship and the direction of the relationship between the independent variable and the dependent variable. The significance in the spearman test can be seen from the presence of two stars (***) on the strength of the relationship. The strength of the relationship can be seen from the value of the correlation coefficient (r). The correlation coefficient is between 1 and -1. Positive and negative signs indicate the direction of the relationship.

Table 2. Strength and Direction of Correlation between Research Variables.

<table>
<thead>
<tr>
<th>Coefficient Correlation Value</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation Strength</td>
<td></td>
</tr>
<tr>
<td>0.000 – 0.199</td>
<td>Very weak</td>
</tr>
<tr>
<td>0.200 – 0.399</td>
<td>Weak</td>
</tr>
<tr>
<td>0.400 – 0.599</td>
<td>Fair</td>
</tr>
<tr>
<td>0.600 – 0.799</td>
<td>Strong</td>
</tr>
<tr>
<td>0.800 – 1.000</td>
<td>Very strong</td>
</tr>
<tr>
<td>Correlation Direction</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>Have the same direction, the higher xi value the higher yi value.</td>
</tr>
<tr>
<td>Negative</td>
<td>Have the opposite direction, the higher xi value the lower yi value.</td>
</tr>
</tbody>
</table>

Finding

1. Measurement Results from Noise in the Production Line Area

Noise measurement in the ceramic industry is divided into three points on the line production section, measuring with Sound Level Meter (SLM) with the following results.
Table 3. Measurement Results from the Noise Point in the Production Line

<table>
<thead>
<tr>
<th>Measurement Point</th>
<th>Measurement Time</th>
<th>Noise intensity</th>
<th>Duration of Exposure</th>
<th>Types of Noise</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forming</td>
<td>10.45</td>
<td>85.9 dB</td>
<td>8 hours</td>
<td>Continue</td>
<td>&gt; NAB</td>
</tr>
<tr>
<td>Glazing</td>
<td>11.00</td>
<td>80.1 dB</td>
<td>8 hours</td>
<td>Continue</td>
<td>&lt; NAB</td>
</tr>
<tr>
<td>Packing and Loading</td>
<td>11.15</td>
<td>85.3 dB</td>
<td>8 hours</td>
<td>Continue</td>
<td>&gt; NAB</td>
</tr>
</tbody>
</table>

Noise measurement was carried out at each sound source in the PT KIA Ceramic MAS, Gresik line production area. The highest noise was generated from the forming area. The source of noise in this area was caused by the use of GF Box Feeder machines or extruder machines. The measurement of noise intensity in units with continuous noise types was carried out once with a distance of one meter from each sound source, the average noise intensity in each unit was then calculated.

Based on the table, the point of forming and packing and loading has a noise intensity above the threshold value of 85 dB. The highest noise point in the forming area was 85.9 dB.

2. Results of Measurement of Physical Workloads

The physical workload was measured by calculating the 10 pulse method which was then calculated using the calculation of the cardiovascular load (CVL) percentage.

2.1 DNI data were taken by measuring resting pulse before workers begin their work.

2.2 DNK data were taken by measuring the pulse when the workers start doing their work, this include:
   a. DNK Measurement 1 at 08.30 WIB
   b. DNK Measurement 2 at 09.30 WIB
   c. DNK 3 Measurement at 10.30 WIB
   d. DNK 4 Measurement at 11.30 WIB

The results of the measurement of the average time of the 10 pulse workers were included in the 10 pulse method equation that the workers’ pulse per minute was obtained. After this calculation, the physical workload was calculated by the cardiovascular load (CVL) method as shown below:

<table>
<thead>
<tr>
<th>Physical Workload Levels</th>
<th>Frequency (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light</td>
<td>11</td>
<td>31</td>
</tr>
<tr>
<td>Moderate</td>
<td>25</td>
<td>69</td>
</tr>
<tr>
<td>Heavier</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Heavy</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Very heavy</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td>100</td>
</tr>
</tbody>
</table>

The distribution shows that the highest category is moderate physical workload by 25 (69%) workers.

3. Results of Systolic Blood Pressure Measurement

The average systolic blood pressure before working and after working increased, but the categorization of an increase in blood pressure is based on a normal increase and an abnormal increase. Increased systolic blood pressure is in the normal category if the increase is 20 mmHg from the initial measurement. If the increase
exceeds 20 mmHg it is categorized as an abnormal increase.

**Table 5. Distribution of Respondents based on Increased Systolic Blood Pressure of Workers**

<table>
<thead>
<tr>
<th>Increased Systolic Blood Pressure</th>
<th>Frequency (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Increase (£ 20 mmHg)</td>
<td>10</td>
<td>28</td>
</tr>
<tr>
<td>Abnormal increase ( &gt; 20 mmHg)</td>
<td>26</td>
<td>72</td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td>100</td>
</tr>
</tbody>
</table>

A total of 26 (72%) line production workers experienced an increase in systolic blood pressure in the abnormal category before and during work.

4. Relationship of Noise Intensity and Physical Workload to Systolic Blood Pressure

Analysis of the relationship between the intensity of noise and physical workload to an increase in systolic blood pressure was carried out by the following table:

**Table 6. Correlation between noise, physical workload to systolic blood pressure**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Increased Systolic Blood Pressure</th>
<th>Total</th>
<th>Coefficient Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Abnormal</td>
<td></td>
</tr>
<tr>
<td>Noise Intensity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noisy</td>
<td>4 14</td>
<td>25 86</td>
<td>29 100</td>
</tr>
<tr>
<td>Not noisy</td>
<td>6 86</td>
<td>1 14</td>
<td>7 100</td>
</tr>
<tr>
<td>Physical Workload</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light</td>
<td>8 73</td>
<td>3 27</td>
<td>11 100</td>
</tr>
<tr>
<td>Moderate</td>
<td>2 8</td>
<td>23 92</td>
<td>25 100</td>
</tr>
</tbody>
</table>

. Based on table 6, 25 (86%) of workers exposed to noise experienced an increase in systolic pressure, while 1 (14%) worker from the group of workers who were not exposed to noise experienced an increase in systolic pressure.

The results of data analysis using the Spearman rho test (Coefficient Correlation) showed a value of 0.581, which means that noise exposure has a strong relationship and a positive relationship with systolic blood pressure. Therefore, the higher the exposure the higher the risk of increasing systolic blood pressure in the abnormal category. Relationship of noise and systolic blood pressure in ceramics industry workers is significant.

This research is in line with the theory of the mechanism of noise stimulation and blood pressure. The theory states that mechanism of vascular disorders in noisy stimulation can be explained as follows. The noisy stimulated organ has an increased task to continue excitement to the centre. This increase requires energy obtained from glucose metabolic processes. This
metabolic process requires oxygen. Every increase in metabolism in tissue cells is always accompanied by an acute increase in blood flow to the tissues. As a result, there is a reduction in active tone in the vascular wall muscles and contractile properties of the capillary endothelium which cause vasodilation of arteriole, venule, metarteriole, pre-capillary and capillary sphincter(6).

Noise has a stronger relationship to the increase in systole than diastole because systolic pressure tends to be more sensitive to noise than diastolic pressure. Noisy is captured as a stressor by the body which then activates the hypothalamus and then controls the endocrine system, the sympathetic system and the adrenal cortex. Activation of various organs and smooth muscles that are in the control of the sympathetic nervous system such as increasing heart rate is a form of response from the sympathetic nervous system to the impulse of the hypothalamus. The sympathetic nervous system also gives impulses to the adrenal medulla to release epinephrine and norepinephrine to the bloodstream. Both of these hormones have vasoconstrictive effects on blood vessels, causing the heart to pump blood more strongly and an increase in blood pressure in workers. Both of these hormones only affect systolic blood pressure while diastole tends to remain.

Based on table 6, 23 (92%) workers from the physical workload group were experiencing an increase in systolic pressure. A total of 3 (27%) workers from the group with light physical workload experienced an increase in systolic pressure.

The results of data analysis using the Spearman rho test (Coefficient Correlation) showed a value of 0.666 which means that the physical workload relationship has a strong relationship and positive relationship direction to systolic blood pressure. Therefore, the higher the physical workload the higher the risk of increasing systolic blood pressure in the abnormal category. The relationship between physical workload and systolic blood pressure in ceramics industry workers is significant.

The results of this study are in line with the theory stated by Rodahl (1999) in the Tarwaka book (2014) that the higher the category of physical workload the higher the pulse counts. Pulse has a work that is synergistic with blood pressure so that an increase in blood pressure is more likely to occur in workers who have a moderate to high physical workload.(5)

**Conclusion**

Noise above the threshold value allowed in the ceramic production line section occurs at the point of forming and loading packing. Noise exposure has a significant relationship to increased systolic blood pressure. The relationship is strong and positive, so that the higher the noise exposure, the greater the likelihood of an abnormal increase in systolic blood pressure. Most workers have a moderate workload of physical work. Physical workload has a significant relationship to an increase in systolic blood pressure. Relationships that appear are strong and positive. The higher the physical workload the higher the possibility of an increase in systolic blood pressure.

**Funding:** Self – funding

**Conflict of Interest:** There are not any of conflicts amongst the authors

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Determination of Oxidant / Antioxidant Levels in Sheep with Hydatite Cyst in Liver

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Abstract

The larval form of Echinococcus granulosus, hydatid cyst is an important parasitic zoonosis commonly seen in animals and humans. The aim of this study was to investigate the efficacy of oxidative stress on the pathogenesis of cystic echinococcosis with antioxidant / oxidant status in sheep sera. The material of the study was composed of sheep infected with hydatid cyst and healthy cut in slaughterhouse in Van province. The general health status of the sheep before slaughtering was checked by physical examination and blood samples were taken. After slaughtering, cyst hydatid examination was performed in different organs of the animals. The control group consisted of 25 sheep study cases with positive (fertil cyst) and 15 sheep without any pathological lesion on organ examinations and healthy on physical examination. Blood samples taken from animals infected with healthy and hydatid cysts were transported to the laboratory under appropriate conditions and centrifuged. Total antioxidant / oxidant status, paraoxonase and arylesterase activities were measured by using commercial kits. Compared with cystic echinococcosis group and healthy group, the importance was calculated on the TAS, PON1 activities (P <0.01), TOS, ARES activities differences (P <0.001), It decreases. This may cause tissue necrosis and increased inflammation. Decrease in antioxidant reserves; excessive free radical formation in the ewes with cystic echinococcosis causes deterioration of the normal histological structure of the organ in the cyst.

Key words: Cystic Echinococcosis, sheep, PON1, ARES, TAS, TOS,

Introduction

Echinococcus granulosus (E. Granulosus) emerged with cystic echinococcosis the larval development of intermediate hosts to settle in Turkey and the world, is a parasite that threaten human and animal health and zoonoses. In our country, cystic echinococcosis shows a wide spread due to the different distribution of animal species in the regions, climatic conditions and socio-economic development level of the society.

E. Granulosus eggs thrown from the feces which contain adult parasites, cause infection in different types of animals such as sheep, goats and cattle. The disease are known to settle such as organs, especially the liver; kidney, spleen, brain, bone, heart1,2.

For the maintenance of vital and biochemical functions, the balance between pro-oxidants and antioxidants is very important. The deterioration of this balance in favor of pro-oxidants (oxidative stress) can lead to oxidative damage3. However, measuring these markers separately is both time consuming and costly 4. Therefore, total oxidant status (TOS) and total antioxidant status (TAS) are measured and oxidative stress index (OSI) is calculated in recent years5-7.

Paraoxonase 1 (PON1, EC.3.1.8.1) and arylesterase are enzymes encoded by the same gene and have similar active centers. Although the natural substrate of both enzymes is different, the PON1 enzyme has the ability to show both arylesterase and paraoxonase activity by
hydrolyzing phenylacetine, the substrate of arylesterase. In addition, plasma high-density lipoprotein (HDL)-dependent antioxidant enzyme PON1 has been reported to protect low-density lipoprotein (LDL) and HDL against oxidation caused by free radicals and to reduce oxidative stress.

Oxidative stress plays a role in the pathogenesis of various diseases. In parasitic infections, the host creates a reaction mechanism to parasites by free radicals, which cause oxidative stress. There are many studies that show that oxidative stress has occurred in animals infected with parasites.

Significant changes have been reported in blood parameters and host biochemistry of animals exposed to parasitic invasion. In studies conducted with endoparasites infected animals, serum protein, serum globulin, cerulo-plasmin, vitamin A, C, E and B12 and some enzyme and mineral level changes were observed.

This project, total oxidant / antioxidant status, oxidative stress index levels, paraoxonase, arylesterase enzyme activities were investigated in the animals infected with hydatid cyst and the effects of oxidative stress on the pathogenesis of cystic echinococcosis were investigated.

In this project, total oxidant / antioxidant status, oxidative stress index levels, paraoxonase, arylesterase enzyme activities were investigated in the animals infected with hydatid cyst and the effects of oxidative stress on the pathogenesis of cystic echinococcosis were also evaluated.

**Materials and Methods**

**Material**

The material of the study was composed of 2-3 aged Morkaraman sheep brought to Özalp slaughterhouse in Van province. The pre-cut sheep’s general health conditions were checked by physical examination and blood samples were taken. After harvesting, cyst hydatid examination was performed in different organs of the animals. The experimental group of the 25 sheep study, positive for the protoscolex (fertile cyst), the control group consisted of 15 sheep who had no pathological lesion in their organ examination and who were healthy in physical examination. Blood samples were transferred to the laboratory at + 4°C and their sera were removed by centrifugation at 3000 rpm and + 4°C for 10 minutes.

**Method**

Total antioxidant status in serum samples of sheep blood, total oxidant status levels, and paraoxonase, arylesterase, activities “Rel Assay Diagnostics-Bursa / Turkey” brand autoanalyzer using commercial kits (Selectra proxl Clinical Chemistry System-The Netherlands) were determined.

**Statistical Analysis**

SPSS 16.0 Windows program (SPSS Inc., Chicago, IL) was used for statistical analysis of the data. Independent t test was used to determine the differences between the groups and the results were given as mean ± SE (standard error). P <0.05 was considered significant for statistical significance.

**Results & Discussion**

At the completion of the project, the averages of the measured values were tabulated and evaluated statistically. The difference between the control and cystic groups’ mean values of TAS and PON1 (p≤ 0.01), TOS and ARES levels (p≤ 0.001) were found to be significant.

**Table 1. Serum TAS, TOS, PON1 and ARES levels of cystic echinococcosis and healthy sheep.**

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>GROUP</th>
<th>X±SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAS (mmol trolox</td>
<td>Hydatid</td>
<td>1.30±0.04</td>
<td>0.01</td>
</tr>
<tr>
<td>Equivalent/L)</td>
<td>cyst</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>1.42±0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOS (μmol H2O2</td>
<td>Hydatid</td>
<td>7.21±0.46</td>
<td>0.001</td>
</tr>
<tr>
<td>equivalent/L)</td>
<td>cyst</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>5.81±0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PON1 (U/L)</td>
<td>Hydatid</td>
<td>914.21±79.07</td>
<td>0.01</td>
</tr>
<tr>
<td>cyst</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>1109.84±77.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARES (U/L)</td>
<td>Hydatid</td>
<td>1254.62±91.13</td>
<td>0.001</td>
</tr>
<tr>
<td>cyst</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>1441.43±65.51</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cystic echinococcosis is a parasitic disease that causes significant economic losses in animal husbandry. This disease is more prevalent in human...
and animals in developing and underdeveloped countries, which are engaged in agriculture and animal husbandry, environmental health and preventive medicine services are inadequate\(^{18}\). In our country, due to reasons such as the fact that slaughtering of slaughtered animals cannot be controlled under strict rules, cyst hydatic continues to be an important parasitic infection affecting both animal and human health\(^{19}\).

Hydatid cyst causes phagocytic cell activation in the immune defense system of the host organism and as a result, reactive oxygen products and reactive nitrogen products are released by macrophages and leukocytes of the host organism in response to cyst pathogenicity, and reactive nitrogen products are released\(^{20}\).

There are many indicators for the evaluation of oxidative stress and antioxidant status and different methods of measuring them. However, measuring these markers separately is both time consuming and costly\(^{4}\). Therefore, total oxidant status (TOS) and total antioxidant status (TAS) are measured and oxidative stress index (OSI) is calculated in recent years\(^{6-7}\).

Bakır et al.,\(^{21}\) reported that oxidative stress increased in patients with hydatid cyst. In the same study, serum total antioxidant status, total oxidant status levels and oxidative stress index levels were found to be significantly higher in hydatid cyst patients compared to control, whereas paraoxonase and arylesterase activities were significantly lower in hydatid cyst patients compared to control. In this study, it was found that the difference between TAS, TOS, PON1 and ARES levels of cystic echinococcosis and healthy sheep in terms of TAS, PON1 (P <0.01), TOS and ARES levels (P <0.001) was found to be significant and these findings were consistent with the results reported in the literature.

As a result, paraoxonase and arylesterase activities are decreased due to increased oxidative stress in sheep infected with hydatid cyst. This may cause tissue necrosis and increased inflammation. Decrease in antioxidant reserves causes the formation of excessive free radicals in sheep with cystic echinococcosis and deterioration of the normal histological structure of the organ with cyst.

**Conflict of interest:** Not

**Ethical Clearance:** The study was approved by the ethical committee of the University of Van Yuzuncu Yil / College of Veterinary Medicine.

**Source of Funding:** Nill

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Assessment of Health-Related Quality of Life Among Patients Management Program of Chronic Diseases Type 2 Diabetes Mellitus

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Abstract

Type 2 Diabetes Mellitus (T2DM) has significant adverse effects on health-related quality of life. PROLANIS program is a system of governance of health services and health education for social health insurance participants who suffer from type 2 diabetes mellitus to achieve the optimal quality of life independently. This research aims to measure quality of life in T2DM patient. The research was an analytical non-experimental study according to the patient’s perspective using prospective data collection techniques. The subjects are PROLANIS patient T2DM who comply inclusion and exclusion criteria. The data were collected by using the European Quality of Life 5 Dimensions 5 Levels (EQ-5D-5L) Indonesian questionnaire. Data analysis using EQ-5D preference weight for each health state using Indonesian EQ-5D-5L Value Set. Furthermore, the percentage of the patients’ problems, EQ-5D index (utility) and EQ-5D VAS were calculated. A total of 220 patients were included. Seventy-two different EQ-5D index values were described by the patients. Obtained 37 subjects (16.82%) had no problems. The EQ-5D index value highest is 1.000 ($n = 32$, 14.54%) and EQ-5D index value lowest is -0.384 ($n = 1$, 0.45%). EQ-5D index value has an average is 0.57 (SD 0.32) and EQ VAS value has an average is 72.19 (SD 14.73). This study also confirmed that T2DM remains a critical predictor of health outcomes among patients. Results from this study could be constructive in clinical practice, particularly in the early treatment of T2DM patients where improving HRQoL is still possible.

Keywords: Type 2 Diabetes Mellitus, PROLANIS, Quality of life, EQ-5D-5L

Introduction

World Health Organization (WHO) defines Diabetes mellitus as a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced.[1] The International Diabetes Federation (IDF) has estimated that approximately 592 million adults in worldwide will have diabetes in 2035.[2] Diabetes has an adverse effect on quality of life. Most patients suffer from a variety of long-term complications including micro-vascular complications (e.g. neuropathy, nephropathy and retinopathy) and macro-vascular complications (e.g. myocardial infarction, angina pectoris, stroke and amputation). [3] Besides, the trouble of taking oral antidiabetic agents several times a day, the fear of subcutaneous injection of insulin, and incidents of hypoglycaemia might depress diabetic patients and further reduce Health-Related Quality of Life (HRQoL). [4]

HRQoL is depicted as an individual’s perceived quality of life, demonstrating satisfaction in the domains that are influenced by health status.[5] The concept of HRQoL is frequently used in clinical research for assessing pharmaceutical care and treatment outcomes. Moreover, the literature reports HRQoL as a predictor of optimal health care service utilization.[6–8] HRQoL is a multidimensional construct highlighting a person’s physical, cognitive, emotional, psychological and spiritual eminence towards the current health status.[9, 10] Today, evaluation of the patients’ HRQoL is recognized
as an important area of scientific knowledge, since the concept is related to the notion of health, satisfaction and well-being in the physical, psychological, socioeconomic and cultural spheres.[11]

The EQ-5D-5L is a widely used generic preference based HRQoL questionnaire.[12] It consists of 5 dimensions (mobility, self-care, usual activities, pain or discomfort, and anxiety or depression), equipped with five possible levels of problem. Health states can be described with a 5-digit number, where the first number is the answer of the first question, the second number is the answer of the second question, accordingly. Given that EQ-5D is a preference-based questionnaire, these health states can be converted into a utility index by applying the appropriate formula. This instrument also includes a Visual Analog Scale (VAS) with a range from 0 (worst imaginable health state) to 100 (best imaginable health state).

In Indonesia, one of the new strategies cultivated is the management program of chronic diseases (PROLANIS). PROLANIS was initiated by Social Insurance Administration Organization (BPJS). The main objective of PROLANIS is to diminish the risk of complications and attain a better quality of life by using of effective cost and rational measures. The PROLANIS program is a system of governance of health services and health education for social health insurance members who suffer from hypertension and type 2 diabetes mellitus to accomplish the optimal quality of life independently.[13] Activities in PROLANIS include medical /educational consulting activities, home visit, reminder, club activities and health status monitoring. [14] The aim of the present study was to assessment of HRQoL among PROLANIST2DM.

Materials and Method

Study design, settings and recruitment of subjects

A questionnaire-based cross-sectional survey was carried out to assess the HRQoL of Patient Chronic Disease Management Program (PROLANIS)T2DM who comply inclusion and exclusion criteria attending primary health care Antang, Batua, Jongaya, Tamalanrea, primary health care Makassar City, Indonesia, in August 2017 – January 2018.

Inclusion and exclusion criteria

Patients enrolled in the PROLANIS program, adult patients aging 20 years and above, fasting blood sugar levels of patients> 126 mg/dL for 3 consecutive months, patients have been treated for at least 3 months, with a confirmed diagnosis of T2DM and willing to follow the research by signing the informed consent were included in the study. Patients with irregular or non-routine control schedules (not every month of control), patients with incomplete medical record data, patients in circumstances where it is not possible to fill in questionnaires (unable to speak, see and hear) were excluded from this study.

Ethical considerations

The study protocol was approved by the Health Research Ethics Committee of the Faculty of Medicine, Hasanuddin University No. 146/H4.8.4.5.31/PP36-KOMETIK/2017. Prior to data collection, patients who agreed to participate were explained nature and the objectives of the study and were assured of the confidentiality of the information. Written consent was also taken from the patients prior to data collection.

Instrument/QoL measurement

To measure HRQoL, we chose the EQ-5D instrument, which was developed in 1987 by EuroQol15 research groups and is widely used in domestic studies. [15] EQ-5D is applicable to a wide range of health index values for health status. We used the official EQ-5D-5L Bahasa Indonesia version provided by the EuroQol Group. This translation of EQ-5D-5L was produced using a standardized translation protocol that followed international recommendations.[16]

Data collection

The EQ-5D-5L assesses health status across five dimensions (mobility, self-care, usual activities, pain/discomfort and anxiety/depression) each with five response levels (none, slight, moderate, severe and extreme/unable). For example, state ‘11111’ indicates ‘no problems on any of the five dimensions’, while state ‘54321’ indicates ‘unable to walk about, severe problems washing or dressing, moderate problems doing usual activities, slight pain or discomfort, and no anxiety or depression’. [17] The Indonesian value set index ranges from −0.865 to 1, where 1 represents preferred health, 0 represents death and a score of less than 0 represents health states worse than death.[18] The second part of the EQ-5D consists of a 20 cm visual analogue scale (VAS) with endpoints of 0, representing the worst imaginable health state and 100, representing the best imaginable health state.
state, and 100, representing the best. This is used to record the participant’s perception of his or her quality of life.

**Data Analysis**

Descriptive statistics were used to investigate the distribution of variables among PROLANIS T2DM patients. Continuous EQ-5D preference weight for each health state using Indonesian EQ-5D-5L Value Set. Furthermore, the percentage of the patients’ problems, EQ-5D index (utility) and EQ-5D VAS were calculated.

**Result**

Patient’s demographics

Two hundred and twenty T2DM patients were included in the study. The description of sociodemographic variables and frequency distribution of the respondents are summarized in Table 1.

**Table 1: Characteristics of survey respondents (n=220)**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>79</td>
<td>35.91</td>
</tr>
<tr>
<td>Female</td>
<td>141</td>
<td>64.09</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50 years</td>
<td>20</td>
<td>9.09</td>
</tr>
<tr>
<td>50-59 years</td>
<td>94</td>
<td>42.73</td>
</tr>
<tr>
<td>60-65 years</td>
<td>106</td>
<td>48.18</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>194</td>
<td>88.18</td>
</tr>
<tr>
<td>Unmarried</td>
<td>26</td>
<td>11.82</td>
</tr>
<tr>
<td>Monthly Income (IDR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;RMS*</td>
<td>103</td>
<td>46.82</td>
</tr>
<tr>
<td>&gt;RMS</td>
<td>117</td>
<td>53.18</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Working</td>
<td>159</td>
<td>72.27</td>
</tr>
<tr>
<td>Not working/retired</td>
<td>61</td>
<td>27.73</td>
</tr>
<tr>
<td>Level of last education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elementary</td>
<td>102</td>
<td>46.36</td>
</tr>
<tr>
<td>Junior high school</td>
<td>29</td>
<td>13.18</td>
</tr>
<tr>
<td>Senior high school</td>
<td>52</td>
<td>23.64</td>
</tr>
<tr>
<td>University</td>
<td>37</td>
<td>16.82</td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤5 years</td>
<td>106</td>
<td>48.18</td>
</tr>
<tr>
<td>&gt;5 years</td>
<td>114</td>
<td>51.82</td>
</tr>
<tr>
<td>Other diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>39</td>
<td>17.73</td>
</tr>
<tr>
<td>Hypertension</td>
<td>138</td>
<td>62.72</td>
</tr>
<tr>
<td>Kidney failure</td>
<td>15</td>
<td>6.82</td>
</tr>
<tr>
<td>Heart failure</td>
<td>8</td>
<td>3.64</td>
</tr>
<tr>
<td>No comorbidities</td>
<td>20</td>
<td>9.09</td>
</tr>
</tbody>
</table>

RMS: Regional Minimum Salary, IDR: Indonesian Rupiah
A sample of 220 T2DM patients were investigated in this study. Table 1 shows the socio-demographic and clinical characteristics of these respondents. The average age of the respondents was 57.71 ± 5.6 years, and 64.09% of respondents were female. Most patients had only graduated from elementary (46.36%), were married (88.18%), were working (72.27%), and had an average monthly household income above the regional minimum salary (53.18%). Most respondents (51.82%) were diagnosed with diabetes over 5 years prior to participation and 62.72% diagnosed with hypertension.

**EQ-5D health status**

Seventy-two different EQ-5D index values were described by the patients. The EQ-5D index value highest is 1.000 (n = 32, 14.54%) and EQ-5D index value lowest is -0.384 (n = 1, 0.45%). The EQ-5D index value has an average is 0.567. Within 72 different health states, the majority (n=32, 14.54%) started no problems /difficulties in the first, second, third, fourth and fifth domain respectively (mobility, self-care, usual activities, pain/discomfort and anxiety/depression) of HRQoL.

In 220 patients the average EQ-5D utility index score was 0.54 (SD 0.32). The highest EQ VAS value is 100 (n 20, 9.09%) and EQ VAS values lowest is 50 (n 22, 10%). The EQ VAS value has an average is 72.19 SD ± 14.73 as presented in Table 2.

**Table 2. EQ VAS values**

<table>
<thead>
<tr>
<th>EQ VAS</th>
<th>Frequency n=220</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>20</td>
<td>9.09</td>
</tr>
<tr>
<td>95</td>
<td>5</td>
<td>2.27</td>
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<td>90</td>
<td>13</td>
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<td>89</td>
<td>1</td>
<td>0.45</td>
</tr>
<tr>
<td>88</td>
<td>3</td>
<td>1.36</td>
</tr>
<tr>
<td>87</td>
<td>1</td>
<td>0.45</td>
</tr>
<tr>
<td>86</td>
<td>2</td>
<td>0.91</td>
</tr>
<tr>
<td>85</td>
<td>9</td>
<td>4.09</td>
</tr>
<tr>
<td>82</td>
<td>2</td>
<td>0.91</td>
</tr>
<tr>
<td>80</td>
<td>23</td>
<td>10.45</td>
</tr>
<tr>
<td>78</td>
<td>2</td>
<td>0.91</td>
</tr>
<tr>
<td>76</td>
<td>1</td>
<td>0.45</td>
</tr>
<tr>
<td>75</td>
<td>16</td>
<td>7.27</td>
</tr>
</tbody>
</table>

**Discussion**

HRQoL is a multidimensional construct referring to an individual’s perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards, and concerns. It was revealed that the patients with diabetes had generally a negative impact on the QoL. These findings are in line to the studies whereby HRQoL decreased with disease progression and complications.[19] Another study supposed that diabetes has an adverse effect on quality of life. Most patients suffer from a variety of long-term complications as well as micro-vascular complications and macro-vascular complications. In addition, a decreased HRQoL was reported by patients of other chronic diseases from various areas of Indonesia which further augments that chronic diseases have a negative impact of the HRQoL of the patients.

As part of strategies developed to encounter chronic diseases in Indonesia, PROLANIS has main objective to reduce the risk of complications and achieve a better quality of life with the use of cost-effective and rational. In this study, it was found that PROLANIS members are dissatisfied and has negative impact on the QoL proven by the results of assessment on health status of patients rated EQ-5D index score 0.54 (SD 0.32) and EQVAS values is 60 (n 31, 14.09%). Hence, it indicates that PROLANIS has not been functioned optimally in the primary healthcare. Furthermore, a previous study by Perwitasari et al (2017) in Indonesian T2DM outpatients (n = 86) reported that the EQ-5D index score was 0.75 (SD 0.22) (20). Moreover, on clinical condition, Perwitasari et al (2017) also found that T2DM complications were indeed aligned with decreases in EQ-5D index scores.
Notably, despite the similarities, Perwitasari et al (2017) utilized dissimilar methods to calculate and analyze the data, i.e., the EQ-5D-3L instrument and the Thailand TTO value set, which complicates comparison.[20]

Based on a report from BPJS in 2016, the number of PROLANIS DMT2 members in 2016 in primary health care Makassar city was 1,165 patients. Even though they have joined as PROLANIS members, they were still not able to be serviced on primary health care. It happened as it lacks of socialization about the importance of PROLANIS services so that patients felt this program was useless for them. In addition, the maximum home visit program, drug information services and counselling are not running well due to time constraints. It was also found that some PROLANIS members only see a doctor and take medication without taking part in gymnastics and education. In addition, the factors that trigger a patient’s quality of life to be low may be due to the patient’s lack of controlled lifestyle. Moreover, there is no family or party monitoring it. Most PROLANIS members whose conditions are uncontrolled are those who are old enough and have no relatives or family who can take them to visit the primary health care.

**Conclusion**

The findings concluded that T2DM has a negative effect on HRQoL. From total of 220 patients, obtained 37 subjects (16.82%) had no problems. EQ-5D index value has an average of 0.57(SD 0.32) and EQ VAS value has an average of 72.19. This study also confirmed that T2DM remains a critical predictor of health outcomes among patients. Results from this study could be constructive in clinical practice, particularly in the early treatment of T2DM patients where improving HRQoL is still possible.

**Financial support and sponsorship:** Ministry of Research, Technology and Higher Education of the Republic of Indonesia

**Conflict of Interest:** There are no conflicts of interest.

**Acknowledgement:** We would like to thank Ministry of Research, Technology and Higher Education of the Republic of Indonesia for funding this research and the authors wish to acknowledge the patients for participating in the study and the staff at the primary health care for their support in conducting the study.


Health Costs for Detoxification Benzene Exposure Workers in the Mining Oil and Gas Pt. A Balikpapan

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1Department of Occupational Health and Safety, Public Health Faculty, Airlangga University, Surabaya

Abstract

Benzene (C6H6) is a liquid aromatic hydrocarbon volatile, colorless, flammable. Benzene is a carcinogen nature makes very harmful for health. The purpose of this study was to calculate and determine the cost of detoxification due to exposure to benzene in the Mining and Oil and gas workers Earth PT. A Balikpapan. Based on the consumption of foods rich in enzyme CYP2E1, Sulfation, and Glutathione. The variable in this study is the intake of the effective dose required by each individual to calculate the weight, length of employment (years), worked on average each day (hour), and the working time in a week (days) of the respondents, as well as the measurement of the concentration benzene. The highest food costs to be paid by the respondent for liver detoxification use is 883,872.51 IDR, using egg 3,513.18 £, and use avocado Rp3,475.01 $. Every individual has a number of different costs. It’s dependent on the characteristics of workers, the concentration of benzene in the work environment, and the intake of benzene by workers.

Keywords: Benzene, Detoxification, CYP2E1, Glutathione, Sulfation

Introduction

Benzene is known as carcinogen compound, with physical and chemical properties sweet-smelling, volatile and slightly soluble in water. According to the Agency for Toxic Substances and Disease Registry (ATSDR), dangerous chemicals and toxic contained in the oil content is Benzene, Toluene, Ethylene, Xylene (BTEX), TPH (Total Petroleum Hydrocarbon) and Polycyclic Aromatic Hydrocarbon (PAHs). Of the six chemicals benzene exposure is very serious impact on health1.

Exposure to Benzene is one of the causes of disease aplastic anemia. The incidence of this disease varies between 2 to 6 cases per 1 million population depends on the risk of occupational, geographic variation and environmental influences2. According to Aksoy (1991) Benzene exposure of the body can result in depression of the bone marrow (bone marrow) there by inhibiting the production of blood cells causing anemia (a decrease in red cells), leukopenia (decreased WBCs), and thrombocytopenia or decreased platelets3.

If the exposure benzene cannot be controlled by way of the elimination of the working environment, it takes an effort to eliminate or reduce the effects of benzene exposure at the worker’s body. In order to reduce or eliminate the concentration of toxins in the body needed workers exposed to benzene biotransformation process. The purpose of the biotransformation is to transform toxic non-polar (soluble in fats) into polar (solvent-soluble compounds). Then, was changed to the hydrophilic (soluble in water) that can be excreted from the body. Biotransforms substances or chemicals in the body can occur with the help of enzymes that are specific to a particular compound participate4.

Process biotransforms benzene phase 2 involves a variety of enzymes, such as CYP2E1, Glutathione and Sulfation. The enzyme-enzym many times in many different types of food. CYP2E1 enzyme-rich foods are beef liver, cow’s brain, and salmon. Foods that are rich in content sulfation are eggs, chicken, beef and tuna. While diet foods rich in glutathione are avocado, asparagus, carrots, tomatoes, oranges, and broccoli5.
Enzyme-rich food source for benzene detoxification in the body is widely recognized. But in fact obstacles, one of which is the assumption that states bring managerial providing food for detoxification benzene exposure is relatively expensive.

Based on the exposure, the study aims to calculate the cost required for detoxification purposes of benzene exposure for each worker in Mining and Oil and Gas PT. A Balikpapan.

**Methods and Materials**

This research is an observational descriptive with quantitative approach, To calculate and illustrate the data collected. The calculations in this research are done by using the application number crunchers.

The research location is in mining Oil and Gas PT. A Balipapan. The study population was all employees of chemicals totaling 22. Sampling technique using the total sample, The variable in this study was effective dose intake needed by each individual to calculate the weight, length of employment (years), worked on average each day (hour), and the working time in a week (days) of the respondents, as well as the measurement of the concentration benzene. Measurement of weight using the weighing scale manual method body. Working length measurement, the average work every day, and time work week obtained through in-depth interviews with respondents. As for the measurement of benzene in the work environment using NIOSH 1501 method of measurement with activated carbon charcoal pipe using gas chromatographic techniques.

The formula for calculating the intake of benzene to the worker:

\[
C = \frac{R \times TE \times fe \times dt}{wb}
\]

Then calculate the effective dose per day with the calculation results of non-carcinogen intake (intake) above, using the formula below:

\[
\text{Cost of intake} = \text{Effective dose} \times \text{price per Kg of food}
\]

Price information (in Indonesia):

- 1 Kg liver = £ 50,000
- 1 Kg egg = £ 24,000
- 1 Kg avocado = £ 45,000

Food prices above are approximate prices beef liver, eggs and avocado per kilogram on the market, so: \(\text{BP benzene} = (\text{m liver} \times \text{USD} / \text{kg liver}) + (\text{m eggs} \times £ / \text{kg eggs}) + (\text{m avocado} \times \text{Rp} / \text{Kg avocado})\).

**Findings**

Based on the results of measurements of the concentration of benzene in the mining Oil and Gas PT. A as follows:

**Table 1. Concentrations of Benzene**

<table>
<thead>
<tr>
<th>Location Measurement</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ppm</td>
</tr>
<tr>
<td>Processing Facility B</td>
<td>1:35</td>
</tr>
<tr>
<td>Processing Facility C</td>
<td>2:31</td>
</tr>
</tbody>
</table>

Tabel Based on the above, the concentration of benzene in the processing facility B is 1:35 ppm and processing facilities c is 2:31 ppm.
### Table 2. Ratio Effective in dose of each Foodstuffs (n = 22)

<table>
<thead>
<tr>
<th>Benzene Concentration (mg / m³)</th>
<th>Dose-effective and CYP2E1 (Cow Liver)</th>
<th>Dose-effectiveness Sulfation (Egg)</th>
<th>Dose-effective and glutathione (Avocado)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.3</td>
<td>6.371592941</td>
<td>0.056853317</td>
<td>0.034444305</td>
</tr>
<tr>
<td>4.3</td>
<td>10.26755654</td>
<td>0.091616752</td>
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</tr>
<tr>
<td>4.3</td>
<td>8.117506233</td>
<td>0.07243199</td>
<td>0.043882568</td>
</tr>
<tr>
<td>4.3</td>
<td>8.92894381</td>
<td>0.079672396</td>
<td>0.048269133</td>
</tr>
<tr>
<td>7:35</td>
<td>4.958570373</td>
<td>0.044245007</td>
<td>0.026805622</td>
</tr>
<tr>
<td>7:35</td>
<td>12.86690181</td>
<td>0.114810544</td>
<td>0.069557409</td>
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<tr>
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<td>7:35</td>
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<td>0.088486095</td>
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</tr>
<tr>
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<td>0.119204023</td>
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</tr>
<tr>
<td>7:35</td>
<td>9.120958982</td>
<td>0.081385735</td>
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<tr>
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<td>0.002089062</td>
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<tr>
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<td>14.17289791</td>
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<tr>
<td>7:35</td>
<td>9.897811056</td>
<td>0.088317537</td>
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<tr>
<td>7:35</td>
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<td>0.076844328</td>
</tr>
<tr>
<td>7:35</td>
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<td>0.143395237</td>
<td>0.086875306</td>
</tr>
<tr>
<td>7:35</td>
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<td>0.124080275</td>
<td>0.07517343</td>
</tr>
<tr>
<td>7:35</td>
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<td>0.091127139</td>
<td>0.055208933</td>
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<tr>
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<td>0.066669632</td>
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<tr>
<td>4.3</td>
<td>5.071398166</td>
<td>0.045251762</td>
<td>0.02741556</td>
</tr>
</tbody>
</table>

The calculation results illustrated in the following graph:

**Chart. 1 Ratio Effective in Dose of Each Raw Meal (n = 22)**

Based on table 2 and graph 1 shows that the average concentration of benzene in the workplace is 5.82 mg / m³. The highest dose effects which should be consumed by the respondent for beef liver is 16.07040935 Kg (rounding = 16.07 kg), eggs and avocado 0.086875306 0.143395237 kg.
Table 3. Comparison of Benzene Concentrations in Liver Cost Beef, Egg and Avocado (n = 22)

<table>
<thead>
<tr>
<th>Benzene Concentration (mg / m³)</th>
<th>Cose of Cow Liver (S)</th>
<th>Cose of Egg (S)</th>
<th>Cose of Avocado (S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.3</td>
<td>350437.6118</td>
<td>1392.906274</td>
<td>1377.772214</td>
</tr>
<tr>
<td>4.3</td>
<td>564715.6098</td>
<td>2244.610423</td>
<td>2220.222515</td>
</tr>
<tr>
<td>4.3</td>
<td>446462.8428</td>
<td>1774.58376</td>
<td>1755.302737</td>
</tr>
<tr>
<td>4.3</td>
<td>491091.9096</td>
<td>1951.973701</td>
<td>1930.765319</td>
</tr>
<tr>
<td>7:35</td>
<td>272721.3705</td>
<td>1084.002673</td>
<td>1072.22488</td>
</tr>
<tr>
<td>7:35</td>
<td>707679.5997</td>
<td>2812.858327</td>
<td>2782.296351</td>
</tr>
<tr>
<td>7:35</td>
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<td>3173.298617</td>
<td>3138.820423</td>
</tr>
<tr>
<td>7:35</td>
<td>545418.5867</td>
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</tr>
<tr>
<td>7:35</td>
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<td>2920.498559</td>
<td>2888.767062</td>
</tr>
<tr>
<td>7:35</td>
<td>501652.744</td>
<td>1993.950509</td>
<td>1972.286046</td>
</tr>
<tr>
<td>4.3</td>
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<tr>
<td>4.3</td>
<td>21254.19671</td>
<td>84.48038379</td>
<td>83.56249632</td>
</tr>
<tr>
<td>7:35</td>
<td>779509.3852</td>
<td>3098.364664</td>
<td>3064.700634</td>
</tr>
<tr>
<td>7:35</td>
<td>544379.6081</td>
<td>2163.779646</td>
<td>2140.269972</td>
</tr>
<tr>
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<td>781816.9764</td>
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<td>3073.773106</td>
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<tr>
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</tr>
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<td>1633.405989</td>
<td>1615.658876</td>
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<tr>
<td>4.3</td>
<td>278926.8991</td>
<td>1108.668176</td>
<td>1096.62239</td>
</tr>
</tbody>
</table>

In Table 3, the highest food costs to be paid by the respondent for liver detoxification use is 883,872.51 IDR, using egg 3,513.18 £, and use avocado 3,475.01 £.

**Discussion**

Enzymes are proteins that function as catalysts (plunger) all metabolic activity of chemicals in the body, nothing is done without the use of enzymes as the body of vital elements. There are thousands of enzymes that humans need to survive. One of the activities of the body is known most often spend reserves are detoxification enzymes. Detoxification is the process of draining piles of toxins in the body.
Toxic fat-soluble cannot be excreted without metabolic transformation (detoxification) in the liver so it can be dissolved in water. Liver cells have sophisticated mechanisms to break down toxic substances. These include endogenous substances (produced by the body) and exogenous (obtained from the environment; for example xenobiotics). Each chemical is broken down or metabolized via detoxification pathways in the liver called “phase 1” and “Phase 2” 10.

An enzyme that plays a role in metabolic transformation (detoxification) of them were CYP2E1, Sulfation and Glutathione. CYP2E1 in this study obtained from beef liver, Sulfation obtained from the eggs and Glutathione obtained from avocado.

CYP2E1 plays an important role in the metabolic activation of chemicals such as acetaminophen (APAP), acetone, halothane, ethanol, including benzene. Benzene is metabolized into epoxy benzene by CYP2E1, which spontaneously formed phenol. Phenol later becomes hydroquinone and catechol catalyzed by CYP2E1, which is more toxic than phenol. Oxidation of benzene by CYP2E1 become reactive intermediates is a prerequisite cellular toxicity. Sheets and Carlson observed a decrease in CYP2E1 activity and reduced risk of benzene-induced hematotoxicisitas11,

Sulfation one liver detoxification pathways, especially the phase II detoxification. Sulfation system is important in detoxifying several drugs, food additives, and environmental contaminants12,

Glutathione is the most powerful antioxidant in the body and is the most abundant antioxidant in the liver and is also the main detoxifying agent in the body. If the body does not have enough amino acids and cofactors required to produce glutathione, the toxins accumulate in the fatty tissues, brain and nerve sheaths13,

Glutathione is also needed in many complex steps required to perform the immune response, with reserves that reduced glutathione, the liver cannot effectively detoxify toxins. If you have a low glutathione levels, we do not have cysteine containing sulfur that is adequate for the sulfation process13,

Based on the calculation shows that each individual has a different spending. This is because each individual has a different dose food effect. The effective dose can also be dependent on the amount of inhaled benzene concentration, weight, and length of work. The higher the concentration of benzene in the body, the greater the mass of detoxification for the food it needs. It is in line with previous research which states that the greater the concentration of benzene and duration of exposure, the greater the period of detoxifying substances. This means that the greater the expenditure required to buy food containing the enzyme CYP2E1, Sulfation, and Glutathione14.

**Conclusion**

The researcher concludes that the average concentration of benzene in the workplace is 5.82 mg / m3. The highest dose effects which should be consumed by the respondent for beef liver is 16.0704935 Kg (rounding = 16:07 kg), eggs and avocado 0.086875306 0.143395237 kg kg.

The highest food costs to be paid by the respondent for liver detoxification use is 883,872.51 IDR, using egg 3,513.18 £, and use avocado 3,475.01 £.

Consumption of foods containing enzymes CYP2E1, Sulfation, and Glutathione with the right dose is expected to reduce or even eliminate benzene compounds into the worker’s body.

Every individual has a number of different costs. Case It depends on the characteristics of workers, the concentration of benzene in the work environment, and the intake of benzene by workers15.

**Conflicts of Interest:** Nil.

**Source of Funding:** The source of this research costs from the self.

**Ethical Clearance:** The study was approved by the institutional Ethical Board of the Public Health, Airlangga University.

All subjects were fully informed about the procedures and objectives of this study each subject prior to the study signed an informed consent form.

**References**


Levels of Interleukin-10 in Iraqi Childhood Acute Lymphoblastic Leukemia after Chemotherapy

Fatima Mallalah Mohammed¹, Morooj Ali Fahd², Nabaa Ali Jassim²
¹Biochemistry/ Lecturer, ²Lecturer, Middle Technical University – Technical Institute – Baqubah – Department of Community Health Technique

Abstract

Background:- Acute Lymphoblastic Leukemia (ALL) is a group of heterogeneous lymphoid disturbance that results from a monoclonal proliferation and expansion of immature T or B lymphocyte ancestor in the blood, bone marrow, and other organs, Interleukin-10 (IL-10) is a pleiotropic cytokine produced by type 2 helper cells (Th2), as well as macrophages and monocytes and neoplastic B lymphocytes and normal.

Aim of this Study: - The aim of this study is to estimate the level of IL-10, and its role as an indication of response to chemotherapy treatment.

Experimental part: - A probable group study carry on out at Unit of Oncology / Child Center Teaching Hospital / AL-Iskan / Baghdad city during the period from October 2017 to June 2018. 25 patients with newly diagnosis of ALL. IL-10 levels were measured utilize serological methods included the enzyme-linked immunosorbet assay before and after chemotherapy treatment.

Result: - This study was carried out on (25) children patients (11 male, and 13 female) newly diagnosed with ALL. The age rang (1.2 years – 12 years), the mean of age was (5.3±3.3 years). A significant decrease in total serum protein and interlukine-10 was observed after chemotherapy (p<0.001, 0.001 respectively), and significant increase in GOT, GPT was observed after chemotherapy (P=0.012, 1.001 respectively).

Conclusion: - The results of our study offering the promising clinical utility of IL-10 as markers of response to chemotherapy.

Keywords: patients, chemotherapy, lymphoma, acute Lymphoblastic

Introduction

Interleukin-10 (IL-10) is a pleiotropic cytokine produced by type 2 helper cells (Th2), as well as macrophages and monocytes, and neoplastic B lymphocytes and normal. It is highly symmetric to an open reading frame of EBV called BCRF1, and EBV infection of B cells up regulates IL-10 production. IL-10 production has powerful immunosuppressive effects by inhibition of Th1 type cytokines, inclusive interferon-gamma and interleukin-2¹. IL-10 has a strong stimulating effect on B cells, inclusive proliferation, and differentiation. Interestingly, in cell lines derived from B-cell lymphomas, IL-10 has been set up to serve as an anticrime growth factor²,³. Serum IL-10 levels have been set up to be substantial forecast factors for Hodgkin lymphoma and when assays that discover both human and viral IL-10 are employed, for NHLs⁴.

Childhood acute leukemia is the generally common cancer in children appears 31% of all cancers and about 3250 new situations per year in the US⁵. Numerous breakthroughs in the past 50 years have increased the survivability of the disease to greatest than 80%, but survivor face long term morbidities⁶.
Acute leukemia is the generally popular cancer in children but the causes of the disease in the plurality of situation are not known. About 80% are the precursor B cell in origin (CD19+, CD10+), and this immunophenotype has increased in happening over the past several contracts in the Western world. ALL is a group of heterogeneous lymphoid defect that results from a monoclonal proliferation and widening of immature B or T lymphocyte progenitor in the blood, bone marrow, and other organs. Proliferation and accumulation of leukemic cells outcome in the repression of normal hematopoiesis and include different particularly the lymph nodes, liver, extramedullary sites, spleen, central nervous system, thymus and gonads. The genetic and epigenetic deviation frequent in the childhood leukemia’s are often significant prognostic index and coincidence of several of these are climacteric to modern disease rating protocols.

Many studies have shown unprompted IL-10 gene expression and synthesis in a diversity of bone marrow-derived leukemic cells or peripheral blood. These involve B-cells derived from different lymph proliferative disturbance. Since little is known related IL-10 levels in leukemic before and after chemotherapy treatment, we studied clinical samples of patients with childhood acute Lymphoblastic leukemia (cALL) for IL-10 levels before and after chemotherapy treatment.

**Experimental part**

**Subjects**

The study was carried out on (25) children patients (11 male, and 13 female) newly diagnosed with ALL. The age rang (1.2 years – 12 years), the mean of age was (5.3±3.3 years), and BMI range was (17.87±7.57 kg/m²).

The selected patients were diagnosed and treated in Unit of Oncology / Child Center Teaching Hospital / AL-Iskan / Baghdad city during the period from October 2017 to June 2018 under the supervision of specialist in pediatric/oncologist doctors.

**Anthropometric indices measurements:**

**Determination of Body Mass Index (BMI)**

Determined the Body mass index by dividing body weight in kilogram by the square of her height in meter. The equation used in medicine produce a unit of measure of kg/m².

**Laboratory methods**

**Assay of Total Serum Protein (Biuret method)**

Modified Biuret method was used to determine total serum protein using bovine serum albumin as standard.

**Determination of Some Enzyme Markers:**

Determination of Alkaline Phosphatase (ALP Activity), GOT, and GPT by using (Kit leaner).

**Determination of serum interlikin-10:** measurement of IL-10 by used ELISA kit (IBL company, Germany.

**Results**

The mean age as (5.3±3.3 years), 54% of them were less than 5 years of age, as shown in Table (1).

**Table (1): Demographic characteristics of leukemic children**

<table>
<thead>
<tr>
<th>Variable</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age mean± SD (5.3±3.3 years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5 years</td>
<td>13</td>
<td>54.16</td>
</tr>
<tr>
<td>≥5 years</td>
<td>11</td>
<td>45.84</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>100.0</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>10</td>
<td>41.66</td>
</tr>
<tr>
<td>Female</td>
<td>14</td>
<td>58.34</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>100.0</td>
</tr>
</tbody>
</table>

1- Family history and clinical complications:

Only 5 (20.8%) leukemic children had positive family history of leukemia. The associated clinical problems were distributed as followings: 40% of patients have no associated problems, 28% have fever, 16% have headache, 12% have fever & headache and 4% have anemia, as shown in Figures (1, 2).
2- Anthropometric indexes:

The weight of leukemic children was significantly decreased after chemotherapy (p<0.001). There was a highly significant decrease in BMI of leukemic children after chemotherapy (p<0.001) (Table 2):

**Table (2): Mean ± (SD) levels of anthropometric indexes of leukemic children before and after chemotherapy.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before chemotherapy</th>
<th>After chemotherapy</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (Kg)</td>
<td>18.5±8.6</td>
<td>16.7±8.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist circum.(Cm)</td>
<td>58±13.8</td>
<td>56.2±15.1</td>
<td>0.20</td>
</tr>
<tr>
<td>Hip circum.(Cm)</td>
<td>57.3±15.2</td>
<td>59.2±13.1</td>
<td>0.50</td>
</tr>
<tr>
<td>BMI (Kg/m2)</td>
<td>14.5±2.2</td>
<td>13±2.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WHR</td>
<td>1.03±0.17</td>
<td>0.96±0.15</td>
<td>0.30</td>
</tr>
<tr>
<td>WHtR ratio</td>
<td>0.54±0.13</td>
<td>0.56±0.22</td>
<td>0.40</td>
</tr>
</tbody>
</table>

P value less than < 0.05 is significant

3- Total serum protein, liver functions and Cytokine interlukine-10:

A significant decrease in total serum protein and interlukine-10 was observed after chemotherapy (p<0.001, 0.001 respectively), and significant increase in GOT, GPT was observed after chemotherapy (P=0.012, 1.001 respectively) (Table 3):

![Figure (2): Distribution of associated clinical problems.](image-url)
Table (3): Mean ± (SD) levels of total serum protein, Cytokine interleukine-10, GOT, GPT before and after chemotherapy:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before chemotherapy</th>
<th>After chemotherapy</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total serum protein(g/l)</td>
<td>48.77±7.8</td>
<td>31.21±5.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ALK</td>
<td>172.66±82.9</td>
<td>196.5±46.2</td>
<td>0.147</td>
</tr>
<tr>
<td>GOT</td>
<td>32.58±21.76</td>
<td>54.58±29.7</td>
<td>0.012</td>
</tr>
<tr>
<td>GPT</td>
<td>19.04±12.7</td>
<td>52.04±32.91</td>
<td>0.001</td>
</tr>
<tr>
<td>Interlukine-10 (Pg/ML)</td>
<td>66.92±25.09</td>
<td>44.57±29.27</td>
<td>0.001</td>
</tr>
</tbody>
</table>

P value less than< 0.05 is significant

Discussion

1: Anthropometric indexes

The current study showed that weight and BMI of leukemic children was significantly decreased after chemotherapy (p<0.001)(table2). This is comparable to results of previous American study which announced that median 6% weight loss after chemotherapy treatment is reveal among leukemic children\textsuperscript{13}. Our finding is maladjusted with results of Atkinson et.al, study which present that weight and BMI of leukemic children increased after chemotherapy\textsuperscript{14}. Other studies have expounded that children after ALL chemotherapy treatment have a slope to be overweight. However, the generality of the authors confirms the requirement for many surveillance for the developmental disorder of childhood after ALL chemotherapy\textsuperscript{15}. Another study acupest out that also a short-term time of monitoring does not permit for a whole estimate of the danger of obesity-related disturbance in children after chemotherapy treatment\textsuperscript{16}

2: Total serum protein

Serum total protein is a biochemical examination for measured the quantity of total protein in serum. It’s using for treatment and diagnosis of types for diseases including the liver, kidney, or bone marrow, as well as other nutritional or metabolic disorders\textsuperscript{17}.

Our study showed significantly lower levels of total serum protein in leukemic children after chemotherapy (p<0.001) (table 3). This finding is compatible with results by Oleiwi et.al, study who found that total serum protein of leukemic children was significantly reduced at baseline after chemotherapy in comparison to healthy controls\textsuperscript{18}. Toxic injury in the liver may be proliferating in practice any known style of injury, including necrosis, cholestasis, steatosis, vascular injury, and fibrosis. During chemotherapy, liver injury may do not be inverted hepatotoxic anticancer agent; the physician should also look response to Antiemetic’s, analgesics, antibiotics, or other medications. Pre-existing medicinal problems, tumor, hepatitis viruses, immunosuppression, and other infections, and total parenteral nutrition or nutritional deficiencies; all may be affect a host’s ability to liver injury. Therefore In cancer chemotherapy, the liver injury to a toxic reaction is difficult; however, resolution of dosing is oftentimes made instituted on limit toxicity. So basically hepatotoxicity is of major concern and increase non-hepatic toxicity probably caused by altering hepatic rescue. And so on, systematic information for the hepatotoxic effects of chemotherapy is light, and the technique of injury is decided for a few factors\textsuperscript{19}.

In several types of ALL, total serum protein showed within L1 group patients, no significant differences in the mean levels of total serum protein after 1st and 2\textsuperscript{nd} months of treatment when compared with baseline level, also, within L2 group patients, no significant differences in the mean levels for total serum protein after 1st, 2nd, and 3rd months of treatment when compared with baseline level\textsuperscript{18}.While, a study showed that significant reduction in total serum protein is due to the effect of disease and low intake of protein\textsuperscript{20}. However, some studies inconsistent with the current study which showed that the level of total serum protein not affected or there were a slight decrease in its level due to the effect of corticosteroid which cause increase food intake and metabolism alteration\textsuperscript{21, 22}. Other study show decrease in serum total protein concentrations was observed during the ALL induction therapy\textsuperscript{23}.
levels of interleukine-10:

This study has seen that serum levels of IL-10 changed during chemotherapy treatment and are capable to be inverted well the path of disease. Serum IL-10 ratios were found higher in active stages of cancer (at diagnosis), while the opposite results with decreased IL-10 was seen after chemotherapy treatment (P=0.001) (Table3). These findings indicate that sequent determinations of serum IL-10 in children with acute lymphoblastic leukemia could support to estimate more exactly the stage of disease and response to therapy in personal patients. As far as we know, this study is the only study to compare the levels of IL-10 before and after chemotherapy in Iraqi children with acute lymphoblastic leukemia.

other study showed level of serum IL-10 has been found to essential prognosis factor in some hematologic malignancies24. That elevated in the level of IL-10 may be because its resulting by various cells or by malignant cells of the immune system, inclusive monocytes, B and T lymphocytes, and , macrophages25.

The reasons and the kind of noteworthy variation in serum IL-10 concentration shown between the stage of cancer growth active and the stage of cancer stillness are complicated. The elevated of serum IL-10 before the inception of treatment and in patients who recrudesce able reflect tumor volume, and illness advancement. Other study in previously the IL-10 is releasing by the cells of many cancer kind26,27. On the other hand the surgical excision of the gastrointestinal cancers and colorectal resulted in a significant decreasing of serum IL-1028. On the other hand yet a powerful body of guide that IL-10 sport an important Immune-suppressive role29, letting malignant cells to shun immune monitoring30, and enhance cancer growth and diffusion26.

Conclusion

In this study we noted significant decrease in levels of IL-10 after chemotherapy treatment compare with before chemotherapy, this will be clinical advantage for IL-10 as indication of response to chemotherapy treatment. However, it must be confirm that the patients were not sampled at random. The sample collection was chosen very accurately. We recommend that you study the increase of study samples in addition to the study of factors affecting the activation of immunity.

Conflict of Interest: The authors declare that they have no conflict of interest.

Source of Funding: Self

Ethical Clearance: There is no violation of human rights and environmental pollution

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Correlation of Standing Work Position and Musculoskeletal Disorders (MSDs) Complaints on Rack Frame Bending Section Workers in Informal Industry of Rack Making in Surabaya, Indonesia

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¹Department of Occupational Safety and Health, Faculty of Public Health, Airlangga University, 60155 Surabaya, East Java, Indonesia; ²Department of Occupational Safety and Health, Faculty of Public Health, Airlangga University, 60155 Surabaya, East Java, Indonesia

Abstract

This research was conducted to analyze the correlation between standing work position and complaints of musculoskeletal disorders on rack frame bending workers in the informal industry of rack making in Surabaya, Indonesia. When doing rack bending, standing work position was affected by work position. The jobs forced workers to work with non-ergonomic standing work position. This caused workers to experience complaints on the skeletal system (MSDs) faster. This research was an observational research with cross sectional design. The sample used a total population of 5 workers. Data obtained by interview, measurement of height and weight and observation of work position. The data analysis method used was descriptive statistics. It was known that due to non-ergonomic standing work position, 40% of rack frame bending workers had a moderate and high MSDs risk category while 20% of other workers were in the low category. In addition, it was known that 40% of workers had low and moderate MSDs complaints category and 20% of other workers had high MSDs complaints category. Based on the results, the standing work position on the rack frame bending workers had a strong and positive correlation with a correlation coefficient of 0.655. In addition to the work position, there was a strong correlation between age, working period, and BMI with MSDs complaints of rack frame bending workers. It was recommended that the employer adjust the frame size on each bending machine and provide seat for workers to rest.

Keywords: standing work position, musculoskeletal disorders complaints, REBA, NBM

Introduction

Musculoskeletal complaints are complaints of skeletal muscle parts that are felt by a person starting from a complaint of bearable to unbearable pain. Complaints to this disorder are usually termed musculoskeletal disorders³. According to Peter Vi, there were several factors that can cause musculoskeletal disorders (MSDs) such as excessive muscle stretching, repetitive movements, unnatural working attitudes, secondary causes, and combination factors⁴.

According to the European Survey on Working Condition in 2005, around 24.7% of European workers complained back pain, 22.8% complained muscle pain, and about 45.5% of workers reported working with fatigue while 35% were assigned to handle heavy loads in their work⁵. In Europe, this occupational disease affected millions of workers and can spend billions of euros to fund treatment for workers. Whereas in chronic cases, MSDs can even caused disability which can caused workers to stop working⁶.

Workers in the rack frame bending section in the informal rack making industry in Surabaya, Indonesia, worked with standing work position. This section required workers to work with changing work position while standing, that was stooping and twisting their waist to press and lift the lever so that the rack frame can be bent. The working process of the rack frame bending section was carried out for 7 working hours/day or >50% work shift. Based on observations, it was known that the frequency of repetitive motion in rack frame bending section every 30 seconds was 6 times, and there were
workers who’s complaining pain in some of their limbs.

According to previous research on welders with standing work position, showed that there was a correlation between standing work position and MSDs complaints especially on the neck, back, and shoulders, also the risk of MSDs was included in the moderate category. In addition, based on the results of research conducted on weaving workers at PT. Delta Merlin Textile, Kebakkramat, Karanganyar, known that there was a very strong correlation between standing work position and musculoskeletal complaints and a positive direction of correlation.

Thus, the purpose of this research was to learn the direction and strength of the correlation between working position and musculoskeletal disorders complaints in rack frame bending section workers in the informal industry of rack making in Surabaya, Indonesia.

Material and Method

Based on the data collection method, this research was observational, data obtained by observing without giving any treatment to the objects of research during the research. Based on the analysis, this research was descriptive statistics, which described the process by analyzing the strength and direction of correlation between variables.

The population of this research were all workers in the rack frame bending section in the informal industry of rack making in Surabaya Indonesia, with total of 5 people. Sampling of this research used the total sampling method, namely the sample used in this research were all members of the population. Variables in this research were age, working period, Body Mass Index (BMI), work position and musculoskeletal disorders complaints. The data collected in this research were only primary data obtained by interviews, measurements of height and weight and observation of work position.

Findings

The research results of workers data collection about the workers age, working period, Body Mass Index (BMI), work position and musculoskeletal disorders complaints were obtained by different methods. Age and working period obtained by interview method, while BMI obtained by calculating using formulas related to measurement of height and weight.

Assessment of work position were done using the Rapid Entire Body Assessment (REBA) method. The application of REBA method is intended to prevent the risk of injury to the musculoskeletal system muscle.

Table 1. Frequency Distribution of MSDs Risk Category on Rack Frame Bending Section Workers Using REBA Method

<table>
<thead>
<tr>
<th>MSDs Risk Category</th>
<th>Frequency (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>1</td>
<td>20%</td>
</tr>
<tr>
<td>Moderate</td>
<td>2</td>
<td>40%</td>
</tr>
<tr>
<td>High</td>
<td>2</td>
<td>40%</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>100%</td>
</tr>
</tbody>
</table>

The risk level of MSDs is divided into several categories, namely very low, low, moderate, high and very high risk but in this research there were no workers with very low and very high MSDs risk categories. Therefore, based on Table 1, it was known that the majority of 40% of workers had moderate and high MSDs risk category and 20% of other workers had low MSDs risk category.

For example, the risk level of MSDs on one of the rack frame bending section workers was known in the high category because it got a final score of 10 (high category final score = 8-10). The final score was obtained from the position score of the position of the neck, body, legs, upper arm, forearm, wrist, type of coupling, type of muscle activity (repetitive movements, significant changes in body posture, and unstable body posture during work) and load or workers force which were of course also significant (the position is becoming flexion-extension or getting more non ergonomic). The risk level of high MSDs category certainly required action to improve work posture as soon as possible to minimize and prevent the occurrence of higher musculoskeletal complaints in workers.

The assessment of musculoskeletal complaints in this research used the Nordic Body Map (NBM) method. Nordic Body Map is a method used to assess the severity of the disruption of the musculoskeletal system. This method can be used to know the level of complaints felt by workers on 28 limbs ranging from feeling bearable to unbearable pain. Determining the level of MSDs complaints can be done by seeing the result of the workers’ body map analysis.
Table 2. Frequency Distribution of MSDs Complaint Levels on Rack Frame Bending Section Workers Using NBM Method

<table>
<thead>
<tr>
<th>MSDs Complaints Level</th>
<th>Frequency (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>2</td>
<td>40%</td>
</tr>
<tr>
<td>Moderate</td>
<td>2</td>
<td>40%</td>
</tr>
<tr>
<td>High</td>
<td>1</td>
<td>20%</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>100%</td>
</tr>
</tbody>
</table>

The level of MSDs complaints was divided into 4 categories, namely low, moderate, high and very high, but in this research there were no workers with very high MSDs complaints. Based on Table 2, the majority of 40% workers had low and moderate MSDs complaints, while 20% of other workers have a higher level of MSDs complaints.

Table 3. Correlation between MSDs Risk Category and Complaints Level on Rack Frame Bending Section Workers

<table>
<thead>
<tr>
<th>MSDs Risk Category</th>
<th>MSDs Complaints Level</th>
<th>Association Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Low</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Moderate</td>
<td>1</td>
<td>50%</td>
</tr>
<tr>
<td>High</td>
<td>1</td>
<td>50%</td>
</tr>
</tbody>
</table>

The correlation between standing work position and MSDs complaints can be seen in Table 3. Based on the results of data analysis that produced the table above, it was known that all workers who had a low MSDs risk category had a moderate MSDs complaints. In addition, it was known that the association coefficient value was 0.655 which means that there was a strong and positive direction of correlation that means the higher the risk of musculoskeletal disorders (the risk of work position), the level of musculoskeletal disorders complaints on rack frame bending section workers will also becomes higher. This was in line with research carried out on plywood making that there was a correlation between standing work position and musculoskeletal complaints on plywood makin in Ketapang, Kendal (p=0.001)\textsuperscript{10}

Research result about workers age, working period and Body Mass Index (BMI) with MSDs complaints can be found in the tables below.
Table 4. Correlation between Age and MSDs Complaints Level on Rack Frame Bending Section

<table>
<thead>
<tr>
<th>Age</th>
<th>MSDs Complaints Level</th>
<th>Total</th>
<th>Association Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low (%)</td>
<td>Moderate (%)</td>
<td>High (%)</td>
</tr>
<tr>
<td>15-19</td>
<td>1 100%</td>
<td>0 0%</td>
<td>0 0%</td>
</tr>
<tr>
<td>20-24</td>
<td>0 0%</td>
<td>0 0%</td>
<td>1 100%</td>
</tr>
<tr>
<td>25-29</td>
<td>1 33%</td>
<td>2 67%</td>
<td>0 0%</td>
</tr>
<tr>
<td>Total</td>
<td>2 40%</td>
<td>2 40%</td>
<td>1 20%</td>
</tr>
</tbody>
</table>

Based on the table above, the results showed that all workers (100%) aged 20-24 years were at a high level of MSDs complaints. In that age group was dominant productive age to work, because workers in this age group will try to work for a long time to get bigger salary. According to Oborne (1995) stated that age is the main cause of complaints on muscle, because as we get older or age increases than the muscle strength decreases. Supported by a research stated that MSDs are the most common and symptomatic health problems in middle and old age. The results of the research revealed that the age group with the highest rate of back pain and muscle fatigue was aged 20-24 years for men.

According to the data analysis that has been done, the age and MSDs complaints level on rack frame bending workers had a strong correlation and a positive or same direction of correlation that seen from the association coefficient of two variables by 0.756.

Table 5. Correlation between Working period and MSDs Complaints Level on Rack Frame Bending Section

| Working period | MSDs Complaints Level | Total | Association Coefficient |
|               | Low (%)               | Moderate (%) | High (%) | N (%) |
| <6 years      | 1 50%                 | 0 0%   | 1 50%                    | 2 100% |
| 6-10 years    | 1 33%                 | 2 67%  | 0 0%                    | 3 100% |
| Total         | 2 40%                 | 2 40%  | 1 20%                   | 5 100% |

Based on the table above it was known that workers whose 6-10 working period were mostly 67% of workers with moderate levels of MSDs complaints. According to the data analysis that has been done, working period and the level of MSDs complaints on rack frame bending workers had a strong correlation and a positive or same direction of correlation that seen from the association coefficient of the two variables by 0.607.

This was not in line with the theory which states that when a muscle receives excessive workload that is carried out repeatedly for a long time, complaints will arise due to damage in joints, ligaments and tendons.
So based on this theory, the working period was directly correlated to MSDs complaints. However, based on the results of the data analysis, it was known that the working period of the worker had a strong and positive direction of correlation or in line with the level of MSDs complaints. In accordance with research conducted on informal workers, that there was a relation between working period and MSDs complaints. Based on the results of the research, it was known that workers who experience high MSDs complaints were workers with <5 years working period not workers with a working period of > 5 years.

**Table 6. Relation Between Body Mass Index (BMI) and MSDs Complaints Level on Rack Frame Bending Section Workers**

<table>
<thead>
<tr>
<th>BMI</th>
<th>MSDs Complaints Level</th>
<th>Total</th>
<th>Association coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low (%)</td>
<td>Moderate (%)</td>
<td>High (%)</td>
</tr>
<tr>
<td>Thin</td>
<td>1 (50%)</td>
<td>0 (0%)</td>
<td>1 (50%)</td>
</tr>
<tr>
<td>Normal</td>
<td>1 (33%)</td>
<td>2 (67%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Total</td>
<td>2 (40%)</td>
<td>2 (40%)</td>
<td>1 (20%)</td>
</tr>
</tbody>
</table>

Based on the table above, it was known that workers included in the thin BMI category had complaints of low and high MSDs around 50% each, whereas in the normal BMI category, the majority of workers around 67% had moderate MSDs and the remaining 33% have low MSDs. According to a research, it was said that height, strength, and body mass were also considered could increase the risk of MSDs but the evidence is far from convincing. But based on the results of other studies conducted at maintenance workers PT. Antam Tbk 2014 UBPE, Pongkor, stated that there was a significant relation between BMI and MSDs complaints.

According to the data analysis that has been done, BMI and MSDs complaints level of workers in rack frame bending section have strong and a positive direction of correlation that can be seen from the association coefficient of both second variables by 0.607.

**Conclusion**

The existence of a strong degree of correlation between working position with MSDs complaints on rack frame bending section workers. In addition, the strong and same direction of the correlation (positive) between the characteristics of workers which are age, tenure, and body mass index (BMI) with MSDs complaint level. The advice given to the company is the company should be evaluating the work station that is usually occupied by rack frame bending section workers. If reshaping the work station to minimize the risk of MSDs requires a large amount of money, the employer can adjust the size of the bending machine lever to the size of the rack that is about to be bend by workers (specialization of frame size on each bending machine) to reduce workers to work with hunchback positions that are too low. In addition to reduce MSDs complaints on workers in standing work position, the employer can provide seats for rack frame bending section workers around the bending machine area so that workers can rest to relax tired limbs after work in a standing work position.

**Conflict of Interest:** All authors have no conflicts of interest to declare.

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Ethical Clearance: The research was approved by the institutional Ethical Board of the Public Health Faculty, Airlangga University.

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Evaluation of Quality of Nursing Documentation in Surgical Wards at Baghdad Teaching Hospitals

Haider M. Majeed1, Tahseen R. Mohammed 2, Raja I. Abid 3

1Assistant instructor; 2Instructor; 3Assistant Professor, Fundamentals of Nursing Department, College of Nursing-University of Baghdad

Background: Nursing documentation has been one of the most important functions of nurses. Objectives: To evaluate quality of nursing documents for nursing care at surgical ward and to find out the relationship between demographic characteristic with nursing documents.

Methodology: A descriptive design study was conducted in the period of 1st January 2017 to 15th August 2017. Utilizing a stratified random sampling method (60) nurses working in surgical ward at Baghdad teaching hospitals.

Results: The majority of the study participants were female who accounted for (58.3%) of the total participants while male constituted (41.7%) making a female male ratio of 1.5:1. Most of the study participants (46.7%) were between ages 18 and 27 years old. (71.7) of the nurses were married and the remainder was single. (45%) of the participants had institute graduate. Majority of them (31.7%) were employee (1-5) years in surgical wards, and finally most of nurses (66.7%) have training session in the nursing documentation.

Conclusions: The study showed that nurses have poor nursing documentation in surgical ward and there is no significant association between the nursing documentation with some demographic characters of selected nurses but significant association between the nursing documentation with training course.

Keywords: Evaluation, Quality of Nursing Documentation, Nursing care, Surgical Wards

Introduction

Nursing documentation is considered as an important indicator to develop nursing care. According to patient safety law, nurses have to document nursing interventions[1]. Nursing documented has jointly practical and legal embodiment in client care thus kind documentation and true notify are fundamental to improve efficiency in client care. In any case of the way used to document, the client’s health-care register is a solemn, legal records is client’s patronage specifics[2,3]. Nurse’s ability to script in a pure brief, fair and legally precise way can safely decrease the danger of misunderstanding and passive patient result concerning to bad communication[4, 5]. Nurses have accepted that registration isn’t dismissing from nursing care and it is not permissive.

Methodology and Materials

A descriptive design study carried out to evaluate quality of nursing documentation in surgical wards at Baghdad teaching hospitals. The study was carried out during the period extended from 1st January 2017 to 15th August 2017. The study population included all nursing staff in four selected hospitals. Inclusion criteria for
nurses were having at least 12 month clinical experience and having any educational level degree in nursing. The sample size estimated 70 nurses with pilot study. Then, these nurses selected to participate with stratified random sampling, according to the number of nursing staff employed in each hospital. Then, for evaluate of each nurse’s documents, in four parts of nursing documents, was selected randomly and analyzed. The demographic data of self fill reporting. For evaluate of nursing documents for nursing care four observational checklists were used. These checklists were evaluate four parts of nursing documents including recording vital sign assessment (4 items), recording wound care (dressing) (11 items), recording medication treatment (4 items) and recording intake and output (I & O) of fluids (10 items). The validity of checklists was determined by content validity and after receiving commends from 10 nursing member checklists were revised. The content validity of the instrument was established through a panel of (15) experts. Test- Coefficients for (29) items of nursing documentation for nursing care were(r= 0.83**) . Data were collected between 8.30am to12.30 pm. The data is analyzed by using SPSS version 20.0.

**Results**

This table revealed that (58.3%) of the study samples were females, and most of them were age group (18-27) years old, a high percentage of them were institute graduate (45%), most of them(71.7%) were married, (31.7%) were for (1-5) years were employment in nursing. Majority of them (31.7%) were employee (1-5) years in surgical wards, and finally most of nurses(66.7%) have training session in the nursing documentation.

<table>
<thead>
<tr>
<th>No</th>
<th>Items</th>
<th>MS</th>
<th>Ass</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vital signs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Body temperature</td>
<td>81.7</td>
<td>Good</td>
</tr>
<tr>
<td>3</td>
<td>Pulse rate</td>
<td>46.7</td>
<td>Fair</td>
</tr>
<tr>
<td>4</td>
<td>Respiration rate</td>
<td>56.7</td>
<td>Fair</td>
</tr>
<tr>
<td>5</td>
<td>Blood pressure</td>
<td>51.7</td>
<td>Fair</td>
</tr>
<tr>
<td>6</td>
<td>Total</td>
<td>2.315</td>
<td>Fair</td>
</tr>
<tr>
<td>7</td>
<td>Wound care (Dressing)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Location of wound</td>
<td>23.3</td>
<td>Poor</td>
</tr>
<tr>
<td>9</td>
<td>Size of wound</td>
<td>6.7</td>
<td>Poor</td>
</tr>
<tr>
<td>10</td>
<td>Wound discharge</td>
<td>3.3</td>
<td>Poor</td>
</tr>
<tr>
<td>11</td>
<td>Total</td>
<td>1.60</td>
<td>Poor</td>
</tr>
<tr>
<td>12</td>
<td>Amount of discharge</td>
<td>3.3</td>
<td>Poor</td>
</tr>
<tr>
<td>13</td>
<td>Color of discharge</td>
<td>3.3</td>
<td>Poor</td>
</tr>
<tr>
<td>14</td>
<td>Signs of infection</td>
<td>5.0</td>
<td>Poor</td>
</tr>
<tr>
<td>15</td>
<td>Pain</td>
<td>1.7</td>
<td>Poor</td>
</tr>
<tr>
<td>16</td>
<td>Signs of wound healing</td>
<td>3.3</td>
<td>Poor</td>
</tr>
<tr>
<td>17</td>
<td>Morning</td>
<td>33.3</td>
<td>Poor</td>
</tr>
<tr>
<td>18</td>
<td>Evening</td>
<td>35.0</td>
<td>Poor</td>
</tr>
<tr>
<td>19</td>
<td>Total</td>
<td>1.15</td>
<td>Poor</td>
</tr>
<tr>
<td>20</td>
<td>Drugs administration</td>
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<tr>
<td>21</td>
<td>Drug name</td>
<td>95.0</td>
<td>Good</td>
</tr>
<tr>
<td>22</td>
<td>Route of administration</td>
<td>86.7</td>
<td>Good</td>
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<tr>
<td>23</td>
<td>Dose</td>
<td>73.3</td>
<td>Good</td>
</tr>
<tr>
<td>24</td>
<td>Total</td>
<td>90.0</td>
<td>Good</td>
</tr>
<tr>
<td>25</td>
<td>Time of administration</td>
<td>3.3</td>
<td>Good</td>
</tr>
<tr>
<td>26</td>
<td>Fluid intake &amp; output</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>Oral route</td>
<td>20.0</td>
<td>Poor</td>
</tr>
<tr>
<td>28</td>
<td>IV infusion</td>
<td>8.3</td>
<td>Poor</td>
</tr>
</tbody>
</table>

Table (1): The Mean of Score of Nurses Documentation for Nursing Care at Surgical Wards.
Table (1): The Mean of Score of Nurses Documentation for Nursing Care at Surgical Wards.

<table>
<thead>
<tr>
<th>Item</th>
<th>Sum of squares</th>
<th>df*</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
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<tbody>
<tr>
<td>Nasogastric tube</td>
<td>1.7</td>
<td>5</td>
<td>8.3</td>
<td>54</td>
<td>90.0</td>
</tr>
<tr>
<td>Gastrostomy route</td>
<td>1.7</td>
<td>5</td>
<td>8.3</td>
<td>54</td>
<td>90.0</td>
</tr>
<tr>
<td>Fluid output</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urination</td>
<td>13.3</td>
<td>7</td>
<td>11.7</td>
<td>45</td>
<td>75.0</td>
</tr>
<tr>
<td>Defecation</td>
<td>13.3</td>
<td>7</td>
<td>11.7</td>
<td>45</td>
<td>75.0</td>
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<tr>
<td>Vomiting</td>
<td>13.3</td>
<td>7</td>
<td>11.7</td>
<td>45</td>
<td>75.0</td>
</tr>
<tr>
<td>Chest tube</td>
<td>3.3</td>
<td>4</td>
<td>6.7</td>
<td>54</td>
<td>90.0</td>
</tr>
<tr>
<td>Drain</td>
<td>8.3</td>
<td>1</td>
<td>1.7</td>
<td>54</td>
<td>90.0</td>
</tr>
<tr>
<td>Nasogastric tube 3</td>
<td>5.0</td>
<td>1</td>
<td>1.7</td>
<td>56</td>
<td>93.3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(A.D.): Assessment Degree, M.s=mean of score [(1 – 1.67) = poor (p); (1.67 – 2.34)= Fair(F) ; [(2.34 – 3) = Good (G)]

This finding of this table indicated that the mean of score was poor documentonitems(5,6,7,8,9,10,11,12,13,20,21,22,23,24,25,26,27,28&29),items (2,3,4,14 and15) was fair documentation, and good documentation on the remaining items.

Table (2): The Association between Nurses Documentation for Nursing Care Score and the Demographic Characteristics.

<table>
<thead>
<tr>
<th>Demographic Variables</th>
<th>Sum of squares</th>
<th>df*</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
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<tr>
<td>Age groups</td>
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<td></td>
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<tr>
<td>Between Groups</td>
<td>.057</td>
<td>2</td>
<td>.029</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within Groups</td>
<td>60.526</td>
<td>57</td>
<td>1.062</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>60.583</td>
<td>59</td>
<td></td>
<td>.027</td>
<td>.974</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>.022</td>
<td>2</td>
<td>.011</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within Groups</td>
<td>14.561</td>
<td>57</td>
<td>.255</td>
<td></td>
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</tr>
<tr>
<td>Total</td>
<td>14.583</td>
<td>59</td>
<td></td>
<td>.043</td>
<td>.958</td>
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<td>Level of education</td>
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<td></td>
<td></td>
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<tr>
<td>Between Groups</td>
<td>2.268</td>
<td>2</td>
<td>1.134</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within Groups</td>
<td>26.982</td>
<td>57</td>
<td>.473</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>29.250</td>
<td>59</td>
<td></td>
<td>2.395</td>
<td>.100</td>
</tr>
<tr>
<td>Years of employed in hospital</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>4.136</td>
<td>2</td>
<td>2.068</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within Groups</td>
<td>191.114</td>
<td>57</td>
<td>3.353</td>
<td>.617</td>
<td>.543</td>
</tr>
<tr>
<td>Total</td>
<td>195.250</td>
<td>59</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Years of employed in surgical ward</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>3.512</td>
<td>2</td>
<td>1.756</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within Groups</td>
<td>147.088</td>
<td>57</td>
<td>2.580</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>150.600</td>
<td>59</td>
<td></td>
<td>.681</td>
<td>.510</td>
</tr>
<tr>
<td>Training course</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Between Groups</td>
<td>2.070</td>
<td>2</td>
<td>1.035</td>
<td>5.238</td>
<td>.008</td>
</tr>
<tr>
<td>Within Groups</td>
<td>11.263</td>
<td>57</td>
<td>.198</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>13.333</td>
<td>59</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This table indicates that there is no significant association between nurse’s documentation for nursing care score and the demographic characteristics (age, gender, level of education, years of experience in surgical ward and years of employed in hospital).
Discussion

Throughout the course of the data analysis of the current study, the findings show the majority of the study were female who accounted for (60%) of the total participants while male constituted (40%). Most of the study participants (46%) were ages group (18-27) years old, the level of education represented that most of them (38%) were from institute graduate, most of them(74%) were married, (38%) for (1-5) years were employment in nursing, most of nurses(70%) have training special session in the nursing documentation. Majority of them (34%) were employee (1-5) years in surgical ward, and finally

These findings are in line with study done by other researcher who reported that the average age of nurses was 32.40+ 5.58 years and they have a 6.40+ 3.58 years clinical experience .of all, 147(86.8%) nurses were female and 168 (98.8%) of them has a BS degree in nursing.83 (48.8%) nurses working in medical wards and 87(51.2%) were working in surgical wards [8].

These findings agreed with findings obtained from other study, who stated that the majority of participation nurses were female142 (87.6%) ,most of them 104(61.2%) were married their mean of age group was 31.38 years,majority of them (51.2%) were working in surgical wards and almost of them (98.8%) had bachelor of science degree in nursing[9].

Twenty nine questions to assess nurses documentation for nursing care in surgical ward, in order to response to first question of the study table five. This table shows the nursing documentation in four selected parts of nursing documents including recording vital sign, recording wound care ,recording medication treatment, and recording intake and output of fluids ,the total mean of score was poor nursing documentation .Further investigation of results of study revealed the most of items that weren’t recorded by nurses in recording wound care dressing ,location of wound(68%), size of wound (82%),wound discharge (96%),all items related to Amount of discharge, colour of discharge, odor of discharge(96%), signs of wound healing(94%) all items that mention up that weren’t recorded by nurses ,the total mean of score related to wound care was poor.

In recording intake and output of fluids most items that weren’t recorded were including, where not recording fluid take through mouth(64%), intravenous fluid(72%), nasogastric tube and gastrostomy route (88%) all items are absent (88%) all items are absent (88%) all items are absent in nursing documentation. Also recording were absent in fluid output including urination,defecaion,vomiting (74%) ,chest tube, drain (88%) and nasogastric tube (92%) of all items related fluid intake and output not recorded by nursing ,the total of mean of score related to fluid intake and output was poor.

In recording vital sign assessment art most items are recorded the mean of score of vital sign was fair. In recording medication treatment most items are recorded by nurses, the total of mean of score related drugs treatment was good.

This finding was in good agreement with that obtained from other researcher reported that the quality of nurses’ documents was moderate. Further investigation showed that most items that weren’t recorded by nurses in recording nursing report part were including “recording the time of reports” (100%), “recording the response of patients to interventions” (97.9%) and “recording the time of nursing cares” (96.5%). In recording medication treatment part most items that weren’t recorded were including “respect suitable method for correct errors” (40.6%) and other items were completely respected by nurses. In recording intake and output of fluids most items that weren’t recorded were including recording accurate time of checking I & O of fluids” (100%) and “recording the differences between the intake and output of fluids” (78.3%). In recording vital sign assessment part most items that weren’t recorded were including “recording the location of controlling vital signs”, “recording the unit of temperature”, “the limb used for controlling the blood pressure” (100%) and “the unit of blood pressure” (97.1%) [8].

Another study agree with the finding of the study who stated the nursing records showed In the vital sign section, data showed that all of them had moderate level and their mean score were 10.69 ± 0.52. In I&O fluid section data showed that 18.6% of flow sheets had moderate quality but most of them 81.4% had suitable quality and their mean score were 13.24 ± 1.07. In chronology sections, all of flow sheets had suitable quality. In drug intervention part, mean score was 11.78 ± 1.42 and most (85.9%) of them had good quality [9].

These findings agreed with study done by other researcher who reported that the quality of nursing care records was poor and inadequate to reflect individualized
nursing care. Their results suggested that more emphasis is needed in nursing practice, and nursing education on the quality of record keeping in order increasing its evidential value [12].

These finding is the same line with study done Rangraz Jedi et al by In one study, assessed the quality of 540 nursing documents and reported that only 11% of these documents didn’t Contain necessary information [13]. Hanifi, (2002) assessed the quality of 30 medical records and reported that only 16.1% of nursing documents had a good quality and 35.8% of them didn’t contain necessary information[14]. Findings of most other studies have also showed that nursing documents have inadequate information about nursing care process and are consistent with the findings of our study [15,16].

In order to respond to second question of the study the association between the nursing documentation for nursing care with some demographic characters of selected nurses. There are no significant relationship between nursing documentation and demographic characteristics of nursing, but significant relationship between nursing documentation and training course.

This finding was in good agreement with that obtained from other study who reported that correlation between age and clinical experience of nurses with quality of their documents chi-square test was used. Results showed that there was no meaningful statistical correlation between qualities of nurses’ documents with their age (χ² = 1.34, df = 2, p = 0.51) [8].

Conclusions

The study concluded that; nurses that working in surgical ward in all selected teaching hospitals had poor documentation in most aspect of nursing daily documentation for nursing care. We recommend conducting teaching programs or sessions must emphasize on all aspects of nursing documentation, for improving quality of nursing documentation and also the study recommends to nursing documentation must be covered widely and in-depth in nursing curriculum of nursing schools.

Acknowledgement: This research was funded by Author. Moreover, we would like to thank the study participants and data collectors for their fully participation and responsible data collection.

Conflict of Interest: None declared.

Ethical approval: The study was approved by the Institutional Ethics Committee.

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The Effect of Empowerment Program on Participation of Mothers with Premature Infants Hospitalized in Neonatal Intensive Care Unit of Sayyed Shirazi Hospital in Gorgan, in 2018

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Abstract

Introduction & Aim: The birth of a premature infant who needs to be admitted to neonatal intensive care unit from birth impedes early communication and participation of mother in her infant’s care. The best way to prevent infant’s harm in hospital is to participate in the care of infant. The purpose of this study was to determine the effect of empowerment program on the participation of mothers with premature infants admitted to neonatal intensive care unit of Sayyed Shirazi hospital in Gorgan, Iran.

Materials and Methods: This study was a randomized clinical trial in 2018, which was conduct on a sample of mothers with premature infant who had randomly been divided into two intervention and control groups. The data collection tools were the Parents’ Participation Inventory designed by Melnick in 1994. Data were analyzed by descriptive statistics (tables, mean and standard deviation) and inferential statistics (independent t-test, paired t-test and covariance) using SPSS-16 software.

Results: There was no significant difference in the level of mothers’ participation in the care of their infants between the two groups before the intervention (p = 0.45). But after the empowerment training program, the results showed that mothers’ participation in the intervention group (19.10 ± 3.09) was higher than the control group (16.8 ± 2.01), (p = 0.01).

Conclusion: The results of this study showed that implementation of empowerment training program increases mothers’ participation in the care of their premature infants. Early intervention and the use of written information along with the booklet will increase the mothers’ participation in the care of their infant.

Key words: Empowerment, Participation, Mothers of premature infants

Introduction

Infants who are born before 37 weeks from the last menstruation day are called premature infants that often weight less than 2500 grams (1). Premature or gestational age of less than 37 weeks is one of the most important health indicators in any society, and infant survival is directly related to gestational age and birth weight (2). Advances in technology and neonatal care have ensured the survival of pre-term infants and reduced their mortality rate (3, 4). Every year 140 million infants are born in the world, of whom around 15 million are premature, accounting for 98% of infant mortality in developing countries (5, 6). Unfortunately, despite all efforts made to prevent preterm births, the prevalence of preterm and low weight infant are still high (1). Various

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factors are involved in preterm birth, including maternal and fetal factors, etc. Maternal factors include maternal illness, chorioamnionitis, multiple birth, maternal smoking, placenta abruption and previa, uterine problems, etc (5). It has been recognized that premature infants require sophisticated treatment and care due to their physical, mental and psychological problems (1). Premature infant hospitalization immediately after birth is inevitable (7). Admission of a newborn infant to a neonatal intensive care unit (NICU) is a frightening and stressful experience for parents (8, 9). Number of adverse outcomes including increased risk of mental problems such as anxiety, depression, traumatic stress disorder, poor parent-infant interactions, and emotional, behavioral and cognitive problems are associated with the premature birth. It has been well documented that, the presence of scary medical equipment in the neonatal intensive care unit and other factors such as unfamiliar staff, restrictive patient policies, infant appearance, vague parental role and parent-infant interactions often create a frustrating and stressful condition for the parents of premature infants (10). As a result, mothers of preterm infants have very different experiences than mothers of term infants and will be challenged and faced with a crisis (11). Birth of a premature infant is not only an emotional and stressful experience for parents, but also an evolutionary stage of life for infant. Although experiencing the motherhood is a joyful one, with the birth of a premature infant the process of mental preparedness to become a mother will be stopped and parents often are not mentally, physically and emotionally ready for such event (6). Understanding the process of becoming a parent is difficult due to the fast and unexpected events that unfold, and having a premature infant can be a sad experience and difficult to cope with and sometime, this emotion is so severe that makes it difficult to control the situation (12). Numerous studies have shown that parental roles and responsibilities will change in these situations (13). Mothers of premature infants are less likely to have parental roles and often have doubts about their abilities to identify and meet their infant’s needs (14). Parents need to be able to cope with these conditions and their new role, but this process is not always easy (13). It is known that the sense of tension is associated with the reduced supportive and protective behaviors and maternal anxiety and behavioral disorder (15). Undoubtedly, the parents of premature infants require support and currently, one of the best and most effective ways to prevent injuries and harm to premature infants is to actively involve their parents in their care (16). Parents of premature infants repeatedly suffer a great deal of psychological distress during their infants’ hospitalization due to lack of involvement and participation in the care process (17). Parents are an inevitable element of a hospitalized child’s care (18). Providing a condition for the presence of parents by their infant will have many benefits for the parents and this is while, most parents are eager to attend and participate actively in the care of their infant (7). When a mother cares for her baby, the sense of motherhood and mother-child solidarity is strengthened. The mother-infant relationship in the intensive care unit is not only as important as the mother-infant relationship for a healthy child, but also is even more important (19). Among suggestions to encourage parents in this situation is to empower them in the care of their infant (17). This type of care was designed by Dr. Bernadette Melinck in 2008 in a form of a program called; “creating opportunities for parent empowerment (COPE), (3). In this program in addition to the psychological support of parents, information about appearance and behaviors of premature infant, parental role, and the care environment are given to parents (20) in order to provide comprehensive care for premature infants and facilitate comprehensive participation of parents in the infant care (19). The empowerment program aims to increase self-awareness, knowledge, motivation, self-esteem, control and preventive behaviors in parents in order to promote health and quality of life (21).

Involving the family in the care and decision-making for their infant from birth is beneficial, as parents are considered primary caregivers and can work closely with the staff and implement the care plan. This type of care has been reported to improve respiratory function, nutrition, weight gain, and reduce hospital stay and treatment costs (22). One of the key components of empowerment considered by health educators is the family participation in care to enhance and improve the quality of life (21). Participation in care enhances family relationships, reduces the negative effects of child illness on the family, maintains family integrity, provides unique care and promotes infant and family health (18). This type of care delivery has many positive outcomes for the neonate such as weight gain, start of oral feeding, self-regulation, neurodevelopment, and family satisfaction (23). Family empowerment is needed for a better life (24) and is one of the main stages of knowledge enhancement and practical participation (24, 25). The purpose of this study was to determine the effect
of empowerment program on participation of mothers with premature infants hospitalized in neonatal intensive care unit of Sayyed Shirazi hospital in Gorgan, Iran.

**Materials and Method**

This study is a randomized clinical trial which was conducted on the two groups of mothers with preterm infants. The setting of this study was Sayyad Shirazi Hospital at the city of Gorgan, Iran. The sample size in this study was determined based on the study of Gavami et al (2012) using G*POWER software with the effect size of 0.94, significant level 0.05, confidence interval of 0.95 and test power 0.080. The samples were randomly divided into two groups of intervention (n=20) and control (n=20). Inclusion criteria were: being over 18 years old, having the ability to read and write, having no history of neonatal intensive care unit care, having no physical or mental illness leading to drug use, having a 26 to 37 weeks old infant who weigh less than 2500 grams, and lack of life-threatening condition in the infant. The simple random sampling method was used in this study.

Data collection tools were demographic information questionnaire (maternal age, education, occupation, number of children) and Parents’ Participation Inventory, which was designed by Melnick in 1997 to measure parental involvement in the neonatal intensive care unit. This questionnaire lists 25 activities that a mother can perform for her infant. Also, the mother is asked to tick every activity that she has performed. The questions in this questionnaire have two options, and the higher number of options selected by the mother indicate the greater care provided by the mother and the more involvement she has in the care of her infant. A score of less than 9 indicates low level of participation, 10-18 indicates moderate level of participation, and score of 19 or more indicates the high level of participation.

In Melnick’s (1997) study, the Cronbach’s alpha of this tool was 0.85.

This project was approved by the Faculty’s Research Council, and a code of ethics was obtained from the University’s Deputy for Technology. After explaining the purpose of study, the necessary permission was obtained from the hospital authorities. According to the inclusion criteria, purposeful sampling was done in the first step and then, the study samples were randomly divided into two intervention and control groups. The researcher, after introducing herself, explained the aims of study to the mothers, ensured them about the principles of confidentiality and anonymity, asked them to provide a written informed consent and informed them that they could withdraw from the study at any time with any reason. The first appointments were made with the participating parents and the meeting place was agreed upon. The interval between each intervention step was 4 days. Three phases of the parent empowerment program were implemented for the parents in the intervention group. The control group received no intervention other than routine care and support in the ward. At the beginning and the end of the study, pre-test and post-test questions were completed by the participants. Data were analyzed by SPSS-16 software.

After the study, audio tapes containing written information and booklets used for parents in the intervention group during the study were given to the control group, so that they could also benefit from the program.

<table>
<thead>
<tr>
<th>No</th>
<th>Session</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>One</td>
<td>Demographic information questionnaire was competed by mothers 4 days after the infant hospitalization and beginning of the intervention in this stage. Then, a 15-minute long audio tape containing information on the first stage along with the booklet was given to the mothers. The information on this stage was related to the appearance and behavioral characteristics of the premature infant, the differences between a pre-term and term infant, environmental characteristics of NICU, and strategies to enhance the maximum participation of parents in the infant care.</td>
</tr>
<tr>
<td>2</td>
<td>Two</td>
<td>It took place 4 days after the first stage. A 15-minute long audio tape containing information on the second stage along with the booklet was given to the mothers. In addition to providing support for the first stage, information on the behavior and evolutionary growth of infant and some suggestion for maximizing the parents’ participation in the care was provided.</td>
</tr>
<tr>
<td>3</td>
<td>Three</td>
<td>It took place one day before the infant discharge. In addition to providing support for the last two stages, information on discharge, how to care for infant at home, how to identify the characteristics of behavior and evolutionary growth of infant and signs of distress were given to parents. After the intervention, mothers completed the post-test.</td>
</tr>
</tbody>
</table>
Findings

The results of independent t-test showed no significant difference between the two groups in terms of mothers’ age (p = 0.62) and length of hospital stay (p = 0.27). In the intervention group, 0.09 of the samples (N=18) were housewives and 0.010 (N = 2) were employed. Also in the control group, 0.080 of the samples (N = 41) were housewives and 0.020 (N = 4) were employed. The result of chi-square test showed no significant difference between the two groups in terms of mother’s occupation (p = 0.66), history of childbirth (p = 0.25), delivery method (p=0.35), history of abortion (p = 0.26), and type of pregnancy (p = 0.36). The Fisher test showed no significant difference between the two groups in terms of education (p = 0.69), and the Mann-Whitney test also showed no significant difference between the two groups in terms of number of children (p = 0.31), (Table 1).

Table 1: Comparison of the mothers with premature infant based on demographic characteristics

<table>
<thead>
<tr>
<th>Demographic characteristics</th>
<th>Type</th>
<th>Intervention</th>
<th>Control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>27.25±4.41</td>
<td>26.65±3.26</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Housewife</td>
<td>18(0.90)</td>
<td>18(80%)</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>Employed</td>
<td>2(0.10)</td>
<td>18(80%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of children</td>
<td>105±1.2</td>
<td>1±0.82</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>2</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>2</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>4</td>
<td>0</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>High school</td>
<td>2</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>University</td>
<td>10</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Level of participation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>10</td>
<td>7</td>
<td>35</td>
<td>0.45</td>
</tr>
<tr>
<td>Moderate</td>
<td>10</td>
<td>13</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>Mean &amp; SD</td>
<td>9.2±3.63</td>
<td>10.35±1.89</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>History of pregnancy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>12</td>
<td>15</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>8</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delivery method</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-section</td>
<td>8</td>
<td>14</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>Virginal</td>
<td>16</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of abortion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8</td>
<td>11</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>12</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length of hospital stay</td>
<td>8.05±3.31</td>
<td>9.25±3.45</td>
<td>0.27</td>
<td></td>
</tr>
</tbody>
</table>

Chi-square test did not show a significant difference between the two groups before and after the intervention (p = 0.45). Also the independent t-test showed no significant difference between the two groups in terms of the mean score of level of participation (p=0.21), (Table 2).
Table 2: Level of parents’ participation in the intervention and control groups before the intervention

Chi-square test showed a significant difference between the two groups in terms of the level of participation after the intervention \((p=0.01)\), so that the level of participation increased to 55% \((N=11)\). Paired t-test showed a significant difference between the two groups in terms of the level of participation before and after the intervention \((p=0.008)\), (Table 3).

Table 3: level of parents’ participation in the intervention and control groups after the intervention

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intervention</th>
<th>Control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>frequency</td>
<td>Percentage</td>
<td>frequency</td>
</tr>
<tr>
<td>Level of participation</td>
<td>Moderate 9 45</td>
<td>16 80</td>
<td>P=0.01</td>
</tr>
<tr>
<td></td>
<td>High 11</td>
<td>55</td>
<td>4</td>
</tr>
<tr>
<td>Mean &amp; SD</td>
<td>19.10±3.09</td>
<td>16.8±2.01</td>
<td>P=0.008</td>
</tr>
</tbody>
</table>

Paired t-test showed a significant difference in the level of participation in the intervention group before and after the intervention \((p<0.01, t=-11.2)\). It also showed a significant difference in the control group \((p<0.01, t=-10.82)\), but the level of participation was more in the intervention group (Table 4).

Table 4: Comparison of the level of parents’ participation in the intervention and control groups before and after the intervention.

<table>
<thead>
<tr>
<th>Time</th>
<th>Intervention group</th>
<th>Control group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before intervention</td>
<td>9.2 ± 3.63</td>
<td>10.35 ± 1.89</td>
<td>P=0.21</td>
</tr>
<tr>
<td>After intervention</td>
<td>19.1 ± 3.09</td>
<td>16.8 ± 2.10</td>
<td>P=0.008</td>
</tr>
<tr>
<td>p-value</td>
<td>p&lt;0.01</td>
<td>p&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>t=11.2</td>
<td>t=10.82</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Covariance test showed a significant difference before the intervention \((p=0.004, \Eta=0.2)\), so that 20% of the changes in parents’ level of participation was due to the empowerment training (Table 5).

Table 5: The effect of empowerment training program on the level of parents’ participation

<table>
<thead>
<tr>
<th>Variance source</th>
<th>Sum of squares</th>
<th>Degree of freedom</th>
<th>Mean of squares</th>
<th>F-value</th>
<th>Significant level</th>
<th>Eta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modified model</td>
<td>70.49</td>
<td>2</td>
<td>35.24</td>
<td>5.4</td>
<td>P=0.009</td>
<td>0.22</td>
</tr>
<tr>
<td>Post-test separator</td>
<td>17.59</td>
<td>1</td>
<td>17.59</td>
<td>2.69</td>
<td>P=0.1</td>
<td>0.06</td>
</tr>
<tr>
<td>Group</td>
<td>63.41</td>
<td>1</td>
<td>63.41</td>
<td>9.71</td>
<td>P=0.004</td>
<td>0.2</td>
</tr>
<tr>
<td>Error</td>
<td>241.4</td>
<td>37.5.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sum</td>
<td>13200</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>311.90</td>
<td>39</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Conclusion

Findings of the present study showed that mothers with premature infants who had an empowerment training program were more involved in the care of their infants. In a study by Melnick et al., four stages of the “creating opportunities for parent empowerment” (COPE) were implemented in the United States (3). In this program, parents learned about the premature infant, family’s abilities and activities that parents can perform. Also, psychological support was provided for parents and sufficient time was given to them for preparation. Consequently, the confidence of parents in caring for their premature infants increased (5). Jaw Brown conducted a study on parents of premature infants that showed that the knowledge of mothers increased after the training. Providing information on infant behavior and interaction with infant reduce mothers’ stress and have positive effects on the level of parents’ participation in the care of their infants (14). Results of studies by Fatemeh Alaei Karahroudi et al. (2012) showed that COPE program had a positive effect on mothers’ participation in the care of their infant and increased it in the intervention group (5). A study by Marzieh Akbarzadeh et al. (2012) showed that mothers’ education increased their positive attitude and self-confidence (26). Study of Noohi et al (2014) revealed that implementation of participatory care and intervention reduces the anxiety of mother more than conventional care (22). Study of Abdolali Zadeh et al. (2015) showed that a health promotion support program is effective in promoting the quality of life in mothers of premature infants (19). Empowering and involving parents and families in the care and decision-making for their infants promote health and wellbeing. People who are able to control their emotions can make the right decision (27). Family empowerment can be an important mechanism in changing care delivery to a better care (10-21). Therefore, empowerment model can be considered as an appropriate model for promoting health, increasing knowledge and enhancing parents’ participation in the care of their infants (22).

Final Result

Considering the results and the impact of empowerment program on the participation of parents in the care of their premature infants, it can be said that the implementation of empowerment program can facilitate active participation of parents in the care of their premature infants. Thus, we suggest to implement this program from the first day of delivery for mothers of premature infants.

Acknowledgement: The present study is part of an MSc dissertation in pediatric nursing with the number: IRCT20170512033932N6, and code of ethics: IR.GOMUS.REC1397.092 approved by Golestan University of Medical Sciences. We would like to express our gratitude to the officials of Golestan University of Medical Sciences and the neonatal intensive care unit of Sayyed Shirazi Hospital in Gorgan.

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In Vitro Technique for Heavy Metal, Cobalt Tolerance in Aloe Vera Callus

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1Biology Department/ College of Science/ Mustansiriyah University-Iraq

Abstract

In vitro tissue culture application in plant biotechnology is an efficient plant propagation tool in arising plants resistant to diverse biotic stresses such as drought and salinity. In this investigation, the aim on the in vitro breeding process applied for plants resistant to heavy metal (HM) stress. The experiment included the following two sequent stages: (i) callus cells initiation, some of which are soma clonal variation with new characteristics, (ii) susceptibility of the callus to HMs as selective factor during multiplication to select variants with enhanced HM-resistance. Aloe callus was grown on media complemented with 0.5, 1.0, 1.5 and 2.0 mg/l cobalt (Co) plus the control treatment. Cobalt accumulation level, mineral status, and callus growth were examined. Results indicate that the increase of Co concentration in medium had influenced callus growth giving the best growth 737 and 56.6 mg for fresh and dry weight respectively at 0.5 mg/l Co concentration, and that growth decreased with increase of Co concentration in medium. With concern to mineral status, cobalt had increased dramatically in callus cells with the increase of Co concentration treatment reaching 108.63ppm at 2.0 mg/l Cobalt, Fe ion concentration reached its highest level 1712.0ppm at 2.0mg/l Cobalt, Mn ions give the highest accumulation level 126.07 ppm at 1.0 mg/l cobalt. While potassium (K) and magnesium (Mg) decreased with increase of cobalt in callus medium recording their highest accumulation level 22244.0 and 492.47 respectively at plant control treatment. Na ions reached its highest level of accumulation 104.3 ppm at 0.5 mg/l cobalt concentration and decreased with the increase of cobalt concentration in the medium.

Key words: Cobalt, tolerance, in vitro, Aloe

Introduction

Soils generally include low levels of heavy metals, high levels can be hazardous to man, plants and animals. In attempt to preserve our environment, new techniques of remediation using chemical, physical and biological theories are being examined (1). Phytoremediation is defined according to Cunningham et al. 1995 (2), as the utilization of plants to withdraw, embrace, or provide harmless environmental contaminants.

Phytoremediation offer many benefits compared to other remediation techniques: it is appropriate to a broad variety of contaminants; organic pollutants could be depraved to CO2 and H2O; it is cost-effective for vast areas having low to moderately contaminated soil surface (3).

Tissue culture is an effective mean that gives the solution to grow millions of cells under controlled conditions, moreover to obtain physiological facts about the action of plant cells under stress conditions (4). Investigate and select at plant cell level established clones of plants with increased tolerance or defiance towards many environmental stresses like drought, heat, salt and heavy metals (5). Plants are often sensitive to both high and low availability of some heavy metal ions as important micronutrients. Useful heavy metals at high level could hurt the soil environment that successively negatively influence soil fertility and plant growth (6).

Bakkaus et al. 2005 (7) mentioned the average Co concentration for plants among 0.1 and 10 µg g⁻¹ dry weight and moreover detailed the beneficial part of Cobalt for the normal metabolic activity of plant at low
concentration. Li et al. 2009 (8) studied toxicity of Cobalt on Hordeum, Lycopersicon and Brassica and reported that Cobalt has reduced growth and biomass in these plants shoots.

This study aimed to establish an in vitro procedure to assess the ability of Aloe vera tissue culture to accumulate heavy metals as an important step for biotechnology researches to select tolerant plants for HMs pollution.

**Materials and Method**

**In vitro culture medium**

Sterilized Explants (leaves) of Aloe vera L. were excised and cultured in universal tubes containing MS medium Murashige and Skoog, 1962 (9). Different concentrations of the Auxin 2,4-D were tested (0.5, 1.0, 1.5, and 2.0 mg/l) which incubated in dark at a temperature 25 ± 1 °C for callus initiation (10).

**Cobalt treatment**

In vitro study was conducted using MS medium complemented with Cobalt, four different concentrations were tested: 0.5, 1.0, 1.5, and 2.0mg/l Cobalt, plus the control treatment, ten replicates were tested for each treatment. Cultures were maintained in a growth chamber at 25 ± 1 °C with a three-week subculture interval.

**Callus growth evaluation**

dry and fresh weight of callus were calculated, using three replicates for each treatment in the Auxin 2,4-D experiment, while regarding the heavy metal experiment using cobalt five replicates were used for each treatment.

**Determination of Co essential and other trace elements content**

Callus samples were previously dried in oven in order to determine the content of Fe, Ca, K, Cu, Mn, Na, Mg, and Co using Atomic Absorption Spectrometer (Solar Mb, thermo Fisher, USA).

**Statistical Analysis**

Biometrical data concerning the callus dry and fresh weight, and the content of cobalt and other chemical elements were subjected to Randomized complete design (CRD) analysis. The least significant difference L.S.D. test was used to study differences means between treatments at P<0.05 (11).

**Results and Discussion**

**Callus initiation**

Table 1 data reveals that dry weight of Aloe callus reached the highest weight (0.110 mg) at 1.0mg/l 2,4-D which differ significantly from the other 2,4-D concentrations treatment and from control treatment, while regarding the fresh weight of callus there was no significant difference between all 2,4-D concentrations treatment except from control.

**Table 1: The effect of 2, 4 D concentration on fresh and dry weight of Aloe vera callus in mg**

<table>
<thead>
<tr>
<th>LSD 0.05</th>
<th>2,4 D mg/l</th>
<th>mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cont.</td>
<td>0.5</td>
</tr>
<tr>
<td>713.5</td>
<td>0.0 b</td>
<td>1241 a</td>
</tr>
<tr>
<td>0.042</td>
<td>0.0 c</td>
<td>0.101 b</td>
</tr>
</tbody>
</table>

**Callus growth on cobalt supplemented media**

Details in table 2 indicate a significant difference in Aloe callus fresh weight at 0.5mg/l cobalt concentration treatment recording 737mg which differ significantly from all except 2.0 mg/l cobalt treatments recording 657 mg, the same result was recorded for dry weight of Aloe callus at 0.5 mg/l cobalt concentration treatment giving 56.6 mg (table 2).
Table 2: Effect of cobalt concentrations on fresh and dry weight of Aloe vera callus in (mg)

<table>
<thead>
<tr>
<th>LSD 0.05</th>
<th>Co concentration</th>
<th>mean</th>
<th>Cont.</th>
<th>0.5</th>
<th>1.0</th>
<th>1.5</th>
<th>2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>122.9</td>
<td>396 b</td>
<td></td>
<td>737 a</td>
<td></td>
<td>509 b</td>
<td>515 b</td>
<td>657 a</td>
</tr>
<tr>
<td>9.37</td>
<td>33.4 b</td>
<td></td>
<td>56.6 a</td>
<td>34.8 b</td>
<td>42.2 b</td>
<td>50.2 ab</td>
<td></td>
</tr>
</tbody>
</table>

Cobalt and other minerals concentration status in callus

Table 3 displays an increase with cobalt concentration in Aloe callus at 2.0 mg/l cobalt concentration treatment giving 108.63 ppm with a significant difference from the other cobalt concentration treatments and that cobalt accumulation decreased and reached its low level at callus and plant control treatments giving 10.27 and 0.0 ppm respectively. In regards to Fe ions, table 3 also shows that the highest accumulation of Fe ions was recorded at 2.0 mg/l cobalt concentration treatment registering 1712.0 ppm which differed significantly from control and the rest of cobalt concentration treatments. Table 3 also demonstrated that Na and Ca ions give the highest accumulation 104.3 and 40363.0 ppm respectively at 0.5 mg/l cobalt concentration treatment which differed significantly from the other treatments, while K and Mg ions decreased with the increase of cobalt concentration in medium reaching its highest levels at plant control treatment which differed significantly from the other treatments. Table 3 also resulted that Cu ions was at their lowest concentration level 0.363 ppm at 0.5 mg/l cobalt treatment.

Table 3: Effect of cobalt concentration on minerals accumulation of Aloe vera callus in (ppm)

<table>
<thead>
<tr>
<th>LSD 0.05</th>
<th>Co concentration</th>
<th>mean</th>
<th>Cont.</th>
<th>0.5</th>
<th>1.0</th>
<th>1.5</th>
<th>2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.88</td>
<td>0.00 f</td>
<td></td>
<td>10.27 e</td>
<td>26.70 C</td>
<td>12.47 d</td>
<td>62.2 B</td>
<td>108.63 a</td>
</tr>
<tr>
<td>24.86</td>
<td>38541.0 b</td>
<td></td>
<td>22029.0 e</td>
<td>40363.0 a</td>
<td>16551.3 f</td>
<td>24669.7 d</td>
<td>26416.0 c</td>
</tr>
<tr>
<td>5.397</td>
<td>492.47 a</td>
<td></td>
<td>12.75 f</td>
<td>233.64 B</td>
<td>113.80 d</td>
<td>91.27 e</td>
<td>143.77 C</td>
</tr>
<tr>
<td>16.51</td>
<td>22244.0 a</td>
<td></td>
<td>12336.0 f</td>
<td>19513.0 b</td>
<td>12956.7 d</td>
<td>12433.3 e</td>
<td>17913.3 c</td>
</tr>
<tr>
<td>0.4489</td>
<td>0.483 e</td>
<td></td>
<td>27.223 a</td>
<td>0.363 E</td>
<td>5.537 c</td>
<td>14.763 b</td>
<td>4.530 D</td>
</tr>
<tr>
<td>1.366</td>
<td>100.70 c</td>
<td></td>
<td>15.81 f</td>
<td>45.21 E</td>
<td>126.07 a</td>
<td>57.34 D</td>
<td>102.37 b</td>
</tr>
<tr>
<td>1.255</td>
<td>287.17 e</td>
<td></td>
<td>502.27 c</td>
<td>613.17 B</td>
<td>146.17 f</td>
<td>365.77 D</td>
<td>1712.0 A</td>
</tr>
<tr>
<td>1.276</td>
<td>45.54 e</td>
<td></td>
<td>61.21 d</td>
<td>104.3 A</td>
<td>20.58 f</td>
<td>70.25 C</td>
<td>101.36 B</td>
</tr>
</tbody>
</table>

Plants capture essential heavy metals like iron, zinc, manganese and copper from the soil due to concentration slope and selective uptakes of these metals (12). The organic and inorganic manures are the agricultural origins of heavy metal contamination, irrigation water; liming, pesticides, and sewage discarding are the major cause of heavy metal release in the soil (13). Chabukdhara et al. (2016) (14) revealed in a case study about heavy
metal in peri-urban and urban-industrial clusters in Ghaziabad, India, that waste water irrigation is behind the heavy load of HMs in agricultural soils. HMs ions strongly affected the activity of many enzymes and cellular metabolism; they also play an important role in protein, photosynthetic pigments, nucleic acids synthesis plus their role in the structural and functional integrity of cell membranes (15).

Cobalt plays an important role in growth and development of plants through regulating plant water utilization and reduce transpiration rate (16). Gad and Hassan (2013) (17) conducted an experiment on tomato with Co application at 7.5 ppm, which improved growth, nutrients level and yield, although Arif et al. (2016) (3) reported that application of cobalt at 5µM on mung beans inhibited seedling growth and caused chlorosis in young leaves.

Hell and Stephan (2003) (18); Thomine and Lanoquar (2011) (19) stated that Fe is easily reduced and oxidized in many biochemical processes and it’s an important cofactor in cytochrome. Cu ions had also an essential role in plant growth by, engaging in many redox-active reactions, while Mn ions act in detoxification of ROS (20). ROS could cause damage of photosynthetic pigments, plants generally response to ROS using antioxidant defense mechanism through synthesis of antioxidant enzymes such as superoxide dismutase, glutathione reductase and catalase that acts as scavengers of ROS (21).

Conclusion

Soil represents the most critical component gathering large amount of dangerous chemical pollutants from different sources per year. And according to Food and Agriculture Organization of UN report (FAO) in 2009, the world population will increase rapidly to reach about 9.6 billion till 2050. Thus, the future world challenge is to obscure the worlds hunger through prolonged agriculture and food production. Plants require some beneficial HMs in a limited quality. While, at high level these metals favor to create distinctive level of toxicity in plant that could inhibit plant growth, damage plant morphology and physiology. This create a need of much expand research on the mechanism of HMs uptake and translocation in relative to their impact on plant growth and development is obligatory to keep step with healthy agronomy production.

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Ethical Clearance: The researchers already have ethical clearance from College of Science, Mustansiriyah University, Iraq.

References


Risk Analysis of H$_2$S Gas Exposure at Benowo Landfill Surabaya

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Abstract

Air pollution can be caused by the number of dangerous gases that arise due to the process of accumulating decaying waste which has acute and chronic effects for people who are exposed. Judging from the data collection method, this study is observational. The study design used in this study was a cross-sectional study design. Risk agent toxicity is expressed in reference doses. For non-carcinogenic inhalation exposures expressed by the Reference Concentration (RfC). Based on primary data derived from the results of measurements of ambient air quality at the loading terminal IIB TPA Garbage Surabaya, the results showed that H$_2$S gas levels of 0.04 where the levels are still below the ambient air quality standards based on East Java Governor Regulation No. 10 of 2009 concerning Quality Standards Air Ambient. From the calculation above, the researcher took the safe value by choosing the cancer risk safe value from the calculation data most likely to be applied by scavengers in the TPA Sampoerna Benowo Surabaya, namely C safe at 3 mg / m$^3$, safe for 3 hours/day and safe for 2 years. Preventive efforts that can be carried out are by controlling the source such as not doing landfill with the open dumping method using the sanitary system. Routinely monitor and control emissions, so that the air quality around the waste picker environment is maintained. Reducing the number of exposures by using personal protective equipment (PPE) in the form of respirators (masks). Perform administrative control by reducing the time and frequency of exposure to H$_2$S dangerous gases. It is better for the Surabaya government and PT X to socialize the hazards and impacts of H$_2$S exposure to workers.

Keywords: Risk Analys, Gas H2S, Risk Management

Introduction

The Waste Management Site is a place where waste reaches the last stage in its management since it starts to rise at the source, collection, transfer or transportation, processing to disposal. Landfill can have an impact on environmental quality such as air pollution from dust and gas resulting from the anaerobic decomposition process, especially if waste disposal uses an open dumping system$^1$.

Direct exposure to environmental pollution, especially gas to health, is more dangerous than indirect exposure. This can be seen from the health problems that occur in landfill waste such as complaints of respiratory problems that occur more on scavengers in TPA garbage, compared to people who are far from pollutant sources.

Air pollution can be caused by the number of dangerous gases that arise due to the process of accumulating decaying waste which has acute and chronic effects for people who are exposed. According to Soemirat (2005) that the decay of waste will produce Methane gas (CH4), Ammonia gas (N3), and Hydrogen Sulphide (H2S) gas which is toxic to the body. States that the gas formation from TPA consists of several types of gas, the gas found from TPA consists mainly of Ammonia(NH3), Carbon dioxide(CO2), Carbon monoxide (CO), Hydrogen(H2),

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Hydrogen sulphide (H2S), Methane (CH4), Nitrogen (N2), and Oxide (O2)2.

H2S gas is included in the type of chemical asphyxiants, namely chemicals that can cause breathing difficulties because the mechanism of toxicity is through chemical reactions or inhibits oxygen transport 3.

**Material and Method**

Judging from the data collection method, this study is observational. The study design used in this study was a cross-sectional study design. Namely, research conducted by collecting exposure and outcome data simultaneously. In a cross-sectional study, researchers only observe phenomena at a certain point 4.

The research design uses a risk analysis paradigm design by taking one of its components, namely risk assessment/risk assessment 5. Risk assessment is used to calculate the estimated level of health risk and determine the control in the form of safe exposure time (Dt). The steps taken in the risk assessment are hazard identification, exposure analysis, dose-response analysis, and risk characteristics.

This research was conducted in 1 (one) work location, precisely at the South IIB waste point in Benowo Surabaya landfill, with the subjects of the study being 37 female respondents aged 15-64 years. The way to determine the sample will be done using Simple Random Sampling. H2S concentration measurements were carried out in the work environment, precisely in the active terminal of the loading and unloading truck using a laboratory test using the Gravimetric method.

The calculation of non-carcinogen intake with sources of exposure through the air can be formulated as follows 6:

\[
I = \text{Intake}, \text{ the amount of risk agent that enters (mg/kg/day)}
\]

\[
C = \text{Concentration risk agent, (mg/m³) for air medium, (mg/L) for drinking water, (mg/kg) for food/food}
\]

\[
R = \text{Rate for air intake (adult: 20 m³/day or 0.83 m³/hour, children: 12 m³/day or 0.5 m³/hour)}
\]

\[
tE = \text{daily exposure time (24 hours/day for residential exposure, 8 hours/day for exposure to the workplace)}
\]

\[
fE = \text{Frequency of annual exposure (Exposure to settlements: 350 days/year) and (Exposure to the work environment: 250 days/year)}
\]

\[
Dt = \text{Duration of exposure, real-time or projections for residential (residential/occupational age), adults: 30 years, children: 6 years}
\]

\[
Wb = \text{Body weight, adult 70 kg / 55 kg (70 kg for Europe from US-EPA 1990, 55 kg for Asia from Nukman, et al. 2005)}
\]

\[
Tavg = \text{Average time period, 30 years x 365 days/year (non carcinogens) or 70 years x 365 days/year (carcinogens)}
\]

The risk characteristics of non-cancer effects can be known by dividing the value of Non-cancerous Intake with the value of RfD or RfC with the following formula 7:

\[
RQ = \frac{Ik}{RFC}
\]

After the RQ value is obtained, then the assumption is used. If the value of \(RQ \leq 1\) indicates no possibility of the risk of noncarcinogenic health effects, it needs to be maintained so that the numerical value of RQ does not exceed 1. While \(RQ > 1\) indicates an indication of the possibility of risk of non-carcinogenic health effects control efforts 8.

After conducting a risk assessment and obtaining results that are beyond the threshold value, the next thing that can be done is risk management. This is done
to minimize and even eliminate the risk of danger arising from the source of danger in the right work. The non-
carcinogenic risk management used is the RQ value. In
reducing the risk agent concentration value, the value of
RQ = 1 so that the intake value = RfC is obtained.

In non-carcinogenic risk management to obtain a
safe value is calculated using the following formula9:

Based on the calculation formula for non-
carcinogenic effects above, it can be derived to find safe
values of C, tE, and Dt as follows:

Findings

a. Risk Analysis and Hazard Identification

H2S is produced due to the process of decomposition
of waste by anaerobic bacteria, in this process, the role of
microbial activity is very important. The microorganisms
that live at this stage are nonmethanogenic types which
consist of facultative anaerobic bacteria. H2S which is
a byproduct of the decomposition of organic matter, the
percentage of H2S gas produced from TPA ranges from
0-0.2%10.

H2S gas is quickly absorbed by the lungs, at low
concentrations, it can cause eye, nose and throat irritation,
at high concentrations it can cause loss of consciousness
and even death 10.

b. Dose Response Analysis

Dose-response analysis is the stage used to
determine the relationship between the size of the dose
or the level of exposure to chemicals with the occurrence
of adverse effects on human health. Where this stage is
a stage to determine the quality of risk agent toxicity has
the potential to have an effect that can be detrimental to
health in at-risk populations.

The risk agent toxicity is expressed in reference
doses. For non-carcinogenic inhalation exposures
expressed by the Reference Concentration (RfC). The
reference dose is used to estimate the amount of exposure
each day in the human population that can be accepted
without causing harmful effects during their lifetime.
The H2S gas RfC value in this study uses the reference
dose value of dust RfC (TSP) in the IRIS list, which is
2.10-3 mg / m3.

c. Exposure Analysis

Based on primary data derived from the results
of measurements of ambient air quality at the loading
terminal IIB At Benowo Landfill Surabaya, the results
showed that H2S gas levels of 0.04 where the levels
are still below the ambient air quality standards based
on East Java Governor Regulation No. 10 of 2009
concerning Quality Standards Air Ambient.

Table 1. Results of Measurement of H2S Levels
in Benowo Landfill in Surabaya

<table>
<thead>
<tr>
<th>No</th>
<th>Parameter</th>
<th>Measured Level</th>
<th>Quality standards</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Hydrogen Sulfide (H2S)</td>
<td>0,01</td>
<td>0,10</td>
<td>µg/Nm3</td>
</tr>
<tr>
<td>2.</td>
<td>Air temperature</td>
<td>34,6</td>
<td>-</td>
<td>°C</td>
</tr>
<tr>
<td>3.</td>
<td>Humidity Nisbi (RH)</td>
<td>62</td>
<td>-</td>
<td>%</td>
</tr>
<tr>
<td>4.</td>
<td>Wind velocity</td>
<td>1,8-6,7</td>
<td>-</td>
<td>m/dt</td>
</tr>
</tbody>
</table>

Table 2. The interview table characteristics of
individual female scavengers at the Benowo Landfill
Surabaya

<table>
<thead>
<tr>
<th>Variable</th>
<th>Average Results</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>tE (Daily exposure time)</td>
<td>9</td>
<td>Hours/day</td>
</tr>
<tr>
<td>fE (Frequency of annual exposure)</td>
<td>336</td>
<td>Day/Year</td>
</tr>
<tr>
<td>Dt (Exposure Duration)</td>
<td>6</td>
<td>Year</td>
</tr>
<tr>
<td>Wb (Weight)</td>
<td>56</td>
<td>kg</td>
</tr>
</tbody>
</table>

In air pollution, the general impact of continuous
exposure to air pollution can cause lung, heart, and other
diseases which are risk factors for death11.

The calculation of carcinogen intake with sources of
exposure through the air can be formulated as follows:

The results obtained through interviews revealed
that the average respondent had a body weight of 56 kg
(Wb), every day worked 9 hours/day (tie), the number
of workdays for one year was 336 days (Fe), and had
worked on an average for 6 years (Dt). With the rate of
inhalation (R) of 20 m³/HR and tag for non-carcinogenic substances is 30 x 365 days/yr. With the results of air measurements that have H₂S (C) concentrations of 0.01 mg / m³, so the amount of noncarcinogenic intake is 5.9 mg/kg.

d. **Risk Characteristics**

Risk characteristics for non-cancer effects can be known by dividing the value of Non-cancerous Intake with the value of RfD or RfC with the following calculations:

Based on the above calculations, it is known that the Ink is 5.9 and the RFC is 0.002. The results of the RQ calculation are 2.95 where the RQ value >1.

According to experts, around the year 2000 the deaths caused by air pollution reached 57,000 people per year. During the 20 years the death rate caused by air pollution rose to close to 14% or up close to 0.7% per year. In addition, material losses caused by air pollution are very large.

e. **Risk Management**

After conducting a risk assessment and obtaining the results that RQ > 1, the next thing that can be done is risk management by carrying out the following calculations:

From the calculation of the figures above, it can be seen that the concentration of H₂S gas at the garbage loading and unloading location even though it is still below the predetermined NAB standard. However, the concentration must be reduced to a maximum concentration of H₂S gas of 3 mg / m³ for the duration of exposure for the next 30 years, assuming that the frequency of workday exposure is 336 days per year and the exposure time is also 9 hours per day.

From the calculation using an average H₂S concentration of 10 mg / m³, we get 3 hours of results, which means that a 56 kg scavenger who is exposed to H₂S gas will be safe for the next 30 years if the daily exposure time is 3 hours/day or around 16 minutes.

From the above calculations, it can be seen that a person weighing 56 kg has been exposed to H₂S gas every day for 9 hours with a dust concentration of 10 mg / m³ so the duration of safe exposure is 2 years.

From the calculation above, the researcher took the safe value by choosing the cancer risk safe value from the calculation data most likely to be applied by scavengers in the At Benowo Landfill Surabaya, namely C safe at 3 mg / m³, safe for 3 hours/day and safe for 2 years.

**Conflicts Of Interest:** All authors have no conflicts of interest to declare.

**Source of Funding:** The source of this research costs from self.

**Ethical Clearance:** The study was approved by Health Research Ethics Committee Faculty of Public Health Airlangga University No: 154-KEPK

All subjects were fully informed about the procedures and objectives of this study each subject prior to the study signed an informed consent form.

**Conclusion**

According to Minister of Manpower Regulation number 5 of 2018, it is explained that H₂S gas exposure can cause upper and eye respiratory irritation, and Nausea. The intake calculation results are 5.9 mg/kg.hari. The results of the current RQ calculation are 2.95 where the RQ value >1, so we have to Safe C calculation results of 3.37 mg/m³, the calculation results are safe for 3 hours/day and the Dt results are safe for 2 years. Risk management has been carried out, but additional control is needed so that the risk of illness and occupational accidents can be reduced.

**Suggestion:**

Preventive measures that can be taken, namely by controlling sources such as not carrying out landfill with the open dumping method using the sanitary system. Carry out regular monitoring and emission control tools so that the air quality around the waste picker environment is maintained. Reducing the number of exposures by using personal protective equipment (PPE) in the form of respirators (masks).

Perform administrative control by reducing the time and frequency of exposure to H₂S dangerous gases. It is better for the Surabaya government to socialize the hazards and impacts of H₂S exposure to workers.

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The Influence of Age, Time off and Pesticides Exposure on Haemoglobin Levels on Vegetable Farmers in Landasan Ulin Utara Subdistrict Banjarbaru City

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Abstract

Several estimates can occur in the status of blood haemoglobin levels, which can be rated lower or higher than average values. Some previous studies revealed several factors that can affect a person’s Hb levels, such as age and level of exposure. Data from the Banjarbaru City Health Office states that 2017 found farmers in Banjarbaru added to anaemia by 25% for the average age of 47 to 67 years. Meanwhile, the average cholinesterase level was 8.36 U / L. In the data it was also found that polycythemia was 13.33% with an average of 48 to 75 years, whereas the average cholinesterase level was 9.33 U / L. This study aim to analyze age, time off and pesticide exposure to hemoglobin levels in vegetable farmers in the Landasan Ulin Utara Sub-District, Banjarbaru City. This type of research is observational analytic with cross-sectional study design. The population in this study were vegetable farmers who used pesticides in the area of Landasan Ulin Utara Sub-District with a sample of 51 people. Based on the results of research related to age and exposure to pesticides or the levels of Acetyl Cholinesterase (AchE) to Hb levels of vegetable farmers in the Landasan Ulin Utara Sub-District, Banjarbaru City. Cholinesterase levels are the most dominant factor.

Keywords: haemoglobin, age, time off, pesticide, vegetable farmer, acetylcholinesterase

Introduction

Haemoglobin has two essential roles in the human body, namely the transport of oxygen throughout the body’s tissues with red blood cells from respiration organs to peripheral tissues and transport of carbon dioxide from peripheral tissues to respiration organs which are then excreted out.1

Several trends can occur in the status of blood haemoglobin levels whose values can be lower or higher than average values. Some previous studies revealed that age factors also influence a person’s haemoglobin (Hb) levels. Adult farmers have a greater risk of chronic effects for anaemia due to inadequate oxygen supply and decreased physical ability and decreased endurance.2 The older a person, the lower of Hb level. The increasing age accompanied by a decrease in health status. There will also be a decrease in lung function which can make it easier for harmful substances to enter through the respiratory system and will be able to enter the lung tissue which then enters the blood vessels that affect the blood levels haemoglobin.3 The high physical activity but not accompanied sufficient rest can increase a metabolic as well as a decrease in pH, where pH is low will reduce the attractiveness between oxygen and haemoglobin.4

Another factor that influences the haemoglobin level of the vegetable farmer is the level of pesticides exposure. The exposure to pesticides could cause the incidence of anaemia in horticultural farmers in Gombong Village, Belik District, Pemalang District, Central Java.5 The exposure to pesticides in Thailand caused farmers as much as 62% to experience Hb levels.6
Data from the Banjarbaru City Health Office states that in 2017, farmers in Banjarbaru found anaemia as much as 25% for the average age of 47 to 67 years. Meanwhile, the average cholesterol level was 8.36 U/L. The data also found 13.33% polycythemia with an average age of 48 to 75 years, while the average cholinesterase level was 9.33 U/L. The data also found 13.33% polycythemia with an average age of 48 to 75 years, while the average cholinesterase level was 9.33 U/L. In the age group of known disorders due to exposure to pesticides haemoglobin form of anaemia was 43.28% from 25% and 28% polycythemia findings of 13.33%.

The findings of anaemia and polycythemia in vegetable farmers in the Banjarbaru area most (55.6%) belong to the category of old vegetable farmers, namely between 40 to 67 years of work rest without a procedural schedule. Banjarbaru is one of the cities that has the most significant number of vegetable production in South Kalimantan, which, in its management, uses pesticides. Therefore, this study will reveal the extent of the influence of age, time off and exposure to pesticides on haemoglobin level in vegetable farmers in the Landasan Ulin Utara Subdistrict, Banjarbaru City.

Materials and Method

The type of this research is observational analytic with a cross-sectional design. Population in this study were vegetable farmers who use pesticides that are in the region of Landasan Ulin Utara Subdistrict. This village has the most number of farmers from all villages in Liang Anggang District and even from all villages in Banjarbaru City, which is equal to 80 vegetable farmers. The number of samples is calculated using the formula Lameshow (1997), which is 51 people.

Findings and Discussion

Table 1. Bivariate Analysis The Influence of Age, Time Off, Cholinesterase and Haemoglobin Levels of Vegetable Farmer in Landasan Ulin Utara Subdistrict, Banjarbaru City

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variable</th>
<th>p-value</th>
<th>β</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin level</td>
<td>Age</td>
<td>0.034</td>
<td>-0.274</td>
<td>0.075</td>
</tr>
<tr>
<td></td>
<td>Time off</td>
<td>0.571</td>
<td>0.075</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Acetylcholinesterase (AchE)</td>
<td>0.002</td>
<td>0.396</td>
<td>0.157</td>
</tr>
</tbody>
</table>

The Influence of Age on Haemoglobin

Based on the results that the p-value of 0.034 (p-value <0.05, which means that there is the influence of age on Hb). The value of β = -0.274, which means that the more age, the lower Hb level will be. The strength of the influence of age on haemoglobin levels of haemoglobin. The value of R² = 7.5%, which means that age affects Hb levels of 7.5%, the remaining 92.5% is influenced by other factors.

In general, the older a person is, the lower the Hb level. With increasing age and decreasing health status, there is a decrease in the function of various organs, including lung function. Decreased lung function makes it easy to organophosphate entering through the respiratory system will be able to enter into the lung tissue further into the blood vessels and affects the haemoglobin concentration in the blood.

At old age, there is a change in the blood which is a decrease in the Total Body Water so that the blood volume decreases and the number of red blood cells (Haemoglobin and Haematocrit) decreases. There is a tendency for the older age of vegetable farmers, the lower the cholinesterase activity in their blood. Cholinesterase is a blood enzyme that is needed so that the nerve can function properly. When cholinesterase is bound, enzymes cannot carry out their duties in the body, especially continuing orders to certain muscles in the body, so that the muscles always move without being able to be controlled.

Old-age farmers have cholinesterase activity which is relatively faster than the age of the respondent especially if it is affected by exposure or exposure to pesticides so
that it can aggravate the occurrence of poisoning. A study conducted by Reddy PB and Jagdish Kanojia in 2012 in India where they found changes in haemoglobin levels, especially a decrease in these levels. The level of haemoglobin is because pesticides reduce production or increase the destruction of red blood cells. It makes the formation of methemoglobin in red blood cells, causing haemoglobin to become abnormal and unable to carry out its function in delivering oxygen.

The Influence of Time Off on Haemoglobin

The results show that p-value is 0.571 (p-value > 0.05, there is no influence of time off on Hb levels). B value = 0.075, which means that the longer the rest period, the higher the Hb level of a person. The strength of the influence of time off is very weak against Hb levels. The value of $R^2 = 0.6\%$, which means a break affecting haemoglobin level of 0.6\%, the remaining 99.4\% is influenced by other factors. It means that statistically, there is no significant relationship between work breaks and impaired haemoglobin levels in vegetable farmers.

Break time does not affect the haemoglobin level because the use of pesticides in a short time can cause poisoning to farmers. Symptoms of chronic organophosphate poisoning arise from inhibition of cholinesterase and will persist for 2 - 6 weeks, resembling mild acute poisoning. However, if exposed again in small amounts, severe symptoms can arise. For carbamate groups, cholinesterase bonds will be temporary and will be re-released in a few hours (reversible), so that chronic poisoning will not arise.

Farmers’ time off from spraying using pesticides is a time estimated that the cholinesterase level would return to normal to 7 days after spraying and no later than 14 days later the cholinesterase level is estimated to increase. If farmers stop using pesticides for a long time, then poisoning due to pesticides will disappear by itself, because the pesticide bonds in the blood will be re-released.

The results of this study are that there is no influence on Hb levels because when taking blood, farmers are not in the period of spraying pesticides so that exposure or exposure to pesticides decreases. As is known that vegetable farmers exposed to anticholinesterase pesticides from the organophosphate group can be repaired. Organophosphate group pesticides can reduce cholinesterase levels in blood serum and erythrocytes. Cholinesterase activity can recover in two weeks without working (spraying) in 1-2 weeks.

The symptoms of organophosphate and carbamate pesticide poisoning usually occur after 4 hours of contact but can occur after 12 hours. The result of the farmer in the village Tejosari shows that the average - average respondent spraying for 2 hours and as much as 76 respondents (97.4\%) spraying <4 hours in every practice spraying.

Based on the provisions stipulated in Permenaker No.Per-03/Men/1986 article 2 paragraph 2a states that in order to maintain unwanted effects, it is recommended that it does not exceed four hours per day in a week in a row when using pesticides. Workers who manage pesticides should not experience exposure more than 5 hours a day and 30 hours a week. While WHO (1996) stipulates the length of spraying of pesticide exposure when working for 5-6 hours per day and every week health tests must be carried out, including blood cholinesterase levels. So it can be said that the more often someone works, the more likely it is to be exposed to pesticides and pesticide poisoning.

Work scheduling and work breaks are work patterns that usually exceed conventional working hours, which are 8 hours a day. Jobs that exceed 8 hours are more at risk for health problems than workers with regular rest periods.

The influence of Acetylcholinesterase (AChE) on Haemoglobin

It was found that the p-value was 0.002 (p-value <0.05, there was an influence of AchE level on Hb levels). B value = 0.396, which means that the higher the AchE level, the higher the Hb level of a person. The value of $R^2 = 15.7\%$, which means that the AchE level affects the Hb level by 15.7\% has a weak influence; other factors influence the remaining 84.3\%. It means there is a statistically significant relationship between exposure to pesticides and the incidence of anaemia in vegetable farmers.

Exposure to pesticides can cause a decrease in oxygen in the tissues. It reduced oxygen being inhaled so that the production of haemoglobin will increase. Every situation that causes a decrease in the transport of the amount of oxygen to the tissue will usually increase the speed of production of red blood cells as compensation for the amount of oxygen transported to the tissues is lacking, the production of red blood cells increases.
The farmer that poisoned by pesticide can be seen by reduced levels cholinesterase in the blood. The cholinesterase will bind class of organophosphate pesticides. The reaction between organophosphate and cholinesterase is called phosphorylase. In the condition of AchE, phosphorylase can no longer be hydrolyzed, which results in AchE buried in receptor sites.

The Influence of Age, Time Off, Cholinesterase and Haemoglobin Levels of Vegetable Farmers in the Landasan Ulin Utara Subdistrict, Banjarbaru City

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient of regression</th>
<th>Sig.</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.270</td>
<td>0.036</td>
<td></td>
</tr>
<tr>
<td>Time off</td>
<td>-0.059</td>
<td>0.644</td>
<td>0.222</td>
</tr>
<tr>
<td>AchE</td>
<td>0.385</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

The results showed that the three independent variables, namely age, time off and AchE levels had an influence on overall Hb levels but the value was only 22.2% while 77.8% was influenced by other factors. Based on the three variables that measured the value of \( \beta \) greatest is 0.385 which levels of AChE means that pesticide poisoning effect on haemoglobin levels where the higher levels of cholinesterase of a person, the higher the haemoglobin level.

Farmers who are poisoned by pesticides are characterised by decreased levels of cholinesterase in the blood because cholinesterase will bind to organophosphate pesticides. Increasing age and declining health status, then a decline in the function of various organs, including lung function. Decreased lung function makes it easy to organophosphate entering through the respiratory tract system will be able to enter into the lung tissue further into the blood vessels and affects the haemoglobin concentration in the blood.

The older the age of the farmer, the more likely direct contact with pesticides. There is a tendency for the older age of vegetable farmers, the lower the cholinesterase activity in their blood. Older farmers have a relatively faster cholinesterase activity compared to younger age respondents, especially if it is influenced by exposure or exposure to pesticides so that it can aggravate poisoning.

Symptoms of organophosphate and carbamate pesticide poisoning usually occur after 4 hours of contact, but can occur after 12 hours. So that the break does not affect the Hb level. Besides, farmers who stop using pesticides for a long time, then poisoning due to pesticides will disappear by itself, because the pesticide bonds in the blood will be rereleased.

Low levels of poisoning in vegetable farmers can occur because, at the time of blood collection, farmers are not in the period of spraying pesticides so that exposure to pesticides decreases. As is known that vegetable farmers exposed to anticholinesterase pesticides from the organophosphate group can be repaired. Organophosphate group pesticides can reduce cholinesterase levels in blood serum and erythrocytes. Cholinesterase activity can recover in two weeks without working (spraying) in 1-2 weeks.

Therefore, repairs can occur if the sprayer is rested for several weeks so that the body can synthesise the cholinesterase enzyme again so that its activity returns to rise. Cholinesterase in plasma takes weeks to return to normal, while in red blood cells, it takes 2 weeks.

Conclusion

There is an influence of age on the haemoglobin of vegetable farmer in the Landasan Ulin Utara Banjarbaru. There was no effect of time off on Hb levels of vegetable farmers in the Landasan Ulin Utara Banjarbaru City. There is an influence of pesticide exposure or AchE level on Hb levels of vegetable farmers Landasan Ulin Utara Banjarbaru City. Pesticide exposure Levels of cholinesterase were the most dominant factor influencing for Hb levels of vegetable farmers in the Landasan Ulin Utara Banjarbaru City.

Ethical Clearance: This research has gone ethical feasibility testing by the Ethical Research Commission of the Faculty of Medicine, University of Lambung Mangkurat.

Source Funding: This study was done by self-funding from the authors.

Conflict of Interest
The authors declare that they have no conflict interests.

References


Impact of Salmonellosis on Antineutrophil Cytoplasmic Antibodies, Alkaline Phosphatase and Lactic Dehydrogenase

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Abstract

The study was carried out during a period of March 2018-July 2019 for the detection of Salmonellosis in 106 suspected patients with age group range from 17 - 69 years, who attended to Baghdad teaching hospitals, that had been examined and defined as suspected cases by specialized physician with the recording of clinical manifestation. The diagnosis done by immunochromatography method, a blood sample was taken from each patients as well as other 30 healthy control matching in age and gender. The study included measurement of the level of antineutrophil antibodies, activity of Alkaline phosphatase, and Lactic acid dehydrogenase in sera of patients and healthy control. The result indicated that anti–salmonella IgM positive in 54 cases, anti–salmonella IgG positive in 40 cases and 12 positive cases with both IgM and IgG. The Level of c-ANCA and p-ANCA, Alkaline phosphatase and Lactic acid dehydrogenase increased significantly, with no statistical difference between the gender of patients sera in comparison with healthy control.

Key Words: Salmonellosis, Alkaline phosphatase, Lactic acid dehydrogenase, c-ANCA, p-ANCA

Introduction

Salmonella typhoid and paratyphoid are transmitted mainly by the fecal-oral route. In most cases an asymptomatic carrier of S. typhi, or an individual who has recently recovered from the infection, continues to excrete large numbers of organisms in the stool and contaminates food or water, either through direct food handling, through transfer of bacteria by flies and other insects, or by contamination of potable water and raw frozen chickens meat

Only 10% of patients recovering from typhoid fever excrete S. typhi in the stool for three months, and in the past 2-3% became permanent carriers. These infections have great potential for epidemic spread. Salmonella typhi infections are commonly mild and self limiting. Severe disease represents the “tip of the iceberg”. In temperate countries persistent carriers are a more important reservoir of infection. A number of host factors increase the risk of Salmonella infections. Disease related (achlorhydria) or iatrogenic (antacids, H2 blockers, proton pump inhibitors) reduction in stomach acidity or gut pathology (surgery, inflammatory bowel disease, malignancy) and recent antibiotics increase the susceptibility to infection. Disease related or iatrogenic immuno suppression and several other infections, notably schistosomiasis, malaria.

The clinical features of typhoid and paratyphoid fever are generally similar, although paratyphoid tends to be a more mild infection. Most patients with enteric fever present with a non-specific gradual onset of an influenza-like illness although Salmonella typhi infection can present with fever and a bewildering array of signs and symptoms ranging from non-metastatic central nervous system syndromes including psychosis and cerebellar ataxia, through to focal involvement of bone, liver, spleen, testes, menninges, vascular prostheses, atheromatous plaques.

In general the enteric fevers are sub-acute infections with an incubation period of approximately 7-14 days (range 3-60 days) following exposure. The illness begins insidiously with non-specific signs and symptoms of fever, headache, muscle and joint aches, malaise, lassitude, anorexia, often a dry cough (sometimes associated with a sore throat). The spleen enlarges, but lymphadenopathy is not usually prominent. Relative bradycardia is considered common in typhoid although
in many series this has not been a feature of the disease. Some abdominal complaints are usual although either diarrhea or constipation may occur.

There is usually some abdominal discomfort, and even in the first week of the disease the patient may notice passage per-rectum of a small amount of blood or melena normal bowel habit is unusual in typhoid. diarrhea (3,18) The clinical evolution of untreated typhoid is divided classically into weeks (19). During the first week the fever rises gradually, and in the second week reaches a high plateau. By the second week the patient has become progressively weaker, has lost weigh. By the third week of infection, if untreated, a dangerous stage is entered upon in which either intestinal perforation or hemorrhage become more likely as the necrotic Peyer’s patches either erode through the wall of the terminal ileum (20) or penetrate a large blood vessels .complications in the third and fourth week also include pneumonia, acute psychosis, coma, myocarditis (21), pericarditis, orchitis, meningitis (22), most salmonellae induce an acute inflammatory response, which can cause ulceration. They may elaborate cytotoxin that inhibit protein synthesis, and contribute to the inflammatory response or to ulceration. However, invasion of the mucosa causes the epithelial cells to synthesize and release various proinflammatory cytokines, including: IL-1, IL-6, IL-8, TNF-α, IFN-α, MCP-1, and GM-CSF. These evoke an acute inflammatory response and may also be responsible for damage to the intestine. Initial host responses involve neutrophil infiltration, followed by the arrival of Lymphocytes and macrophages. Occasionally, diffuse colitis occur mimicking inflammatory bowel disease (23).

Material and Method

The study carried out during the period from (March 2018- July 2019), studied group were involved Suspected patients their age range between 17-69 years. Blood samples were obtained from a total of 106 patients clinically suspected with Salmonellosis that had been examined and defined as suspected cases by specialized physician with the recording of clinical manifestation.

Blood samples

Five mL of venous blood was obtained from each patients and collected in sterilized screw cap plastic tube, blood samples were left for 30 min. at room temperature, then centrifuge at 3000 rpm for five minute, then the serum for each sample was collected in eppendorf tubes and then test for Salmonellosis (IgM and IgG) and stored in deep freeze at -20° C until the time for using.

Immunochromatographic assay

About 100 μl of serum from each sample was added to the sample hole of the kit. The colour density is proportional to the antibody titer. The complexes (appears in colour band after 10 minutes) confirm that the test was performed correctly. This CerTest-salmonella kit which determines the salmonella quality in bloods samples. Pre-coating was achieved to the membrane proceeding to test band region to the monoclonal antibodies of the mouse, it was achieved against salmonella antigens. Through test, samples were reacted with conjugated colors’ (anti-salmonella of monoclonal mouse microsphere (red antibodies)), the samples were dried before that, the combination then travelled to reach membranes via the act of capillaries. While samples move via the membranes tests, tinted particle were migrated. In positive results, certain antibodies that have existed on the membranes captured these particles which lead to appearance of red tinted line that can clearly observed while the other result appears in a green tinted line (the negative results that represent the control samples.

Immunological and Clinical biochemical tests

The level of Anti proteinase-3; Cyclic -anti neutrophil cytoplasmic antibodies (c-ANCA) are examined by Enzyme Linked Immunosorbent Assay (ELISA) according to (24). Alkaline phosphatase and Lactic dehydrogenase Concentration determined according to manufactures instructions of Biosystem (Spain).

Statistical Analysis

The results were analyzed using statistical system SPSS version -18 (T-testing).

Result

Serological tests

Serum level anti –salmonella IgM present in 54 cases with a percent of 50.9%, also, the level of anti –salmonella IgG present in 40 cases with a percent of 37%.While the level of both IgG and IgM present in 12 cases with a percent of 11.3% (Table 1).
Table 1: Distribution of anti-Salmonella IgG and IgM antibodies using immunochromatography method

<table>
<thead>
<tr>
<th>Anti-Salmonella antibodies</th>
<th>Total</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>IgG</td>
<td>106</td>
<td>40</td>
<td>37</td>
</tr>
<tr>
<td>IgM</td>
<td>106</td>
<td>54</td>
<td>50.9</td>
</tr>
<tr>
<td>IgG + IgM</td>
<td>106</td>
<td>12</td>
<td>11.3</td>
</tr>
</tbody>
</table>

Level of p-ANCA and c-ANCA

Level of anti-PR3 in the serum of patients with Salmonellosis shows a significant increasing (P<0.05) in comparison to healthy control, while, the results shows no-significant difference (P>0.05) between the gender in both groups. The serum level of anti-PR3 was (24.82 ± 0.28), (23.64 ± 0.36) in males and females of patients group respectively comparing to healthy control group (15.08 ± 0.92) and (15.85 ±0.15) in males and females respectively as shown in table 2.

Table-2: The level of anti-PR3 (Iu/ml) in sera of Salmonella patients and control

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± SD</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>24.82 ± 0.28</td>
<td>23.64 ± 0.36</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>15.08 ± 0.92</td>
<td>15.85 ± 0.15</td>
<td></td>
</tr>
<tr>
<td>T – test value</td>
<td>0.51</td>
<td>0.91</td>
<td></td>
</tr>
</tbody>
</table>

The level of anti-MPO shows a significant increasing (P<0.05) in serum of patients with Salmonellosis in comparison with healthy control, while the results shows no significant differences (P>0.05) between the gender in both groups. The level was (23.48 ± 0.16), (22.64± 0.22) in males and females of patients groups respectively in comparison with (16.35 ± 0.28), (16.80 ± 0.21) in males and females of healthy control respectively. Table (3).

Table 3: The level of anti-MPO (Iu/ml) in sera of Salmonella patients and healthy control

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± SD</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>23.48 ± 0.16</td>
<td>22.64 ± 0.22</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>16.35 ± 0.28</td>
<td>16.21 ± 0.19</td>
<td></td>
</tr>
<tr>
<td>T – test value</td>
<td>0.55</td>
<td>0.67</td>
<td></td>
</tr>
</tbody>
</table>

Alkaline phosphatase activity

The activity of alkaline phosphatase increased significantly (p≤0.05) in Salmonellosis patients in both gender in comparison with healthy control (Table 4).
Table 4: Alkaline phosphatase activity (Iu/ml) in patients with Salmonellosis and healthy control

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± SD</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>186.37 ± 0.73</td>
<td>179.10 ± 0.45</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1118.58 ± 0.48</td>
<td>115.90 ± 0.39</td>
<td></td>
</tr>
<tr>
<td>T – test value</td>
<td>0.551.42</td>
<td>1.01</td>
<td></td>
</tr>
</tbody>
</table>

Lactic acid Dehydrogenase activity:

The activity of Lactic acid dehydrogenase increased significantly (p≤ .05) in Salmonellosis patients in both gender in comparison with healthy control (Table 5).

Table 5: Lactate dehydrogenase activity (Iu/ml) in patients with Salmonellosis and healthy control

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± SD</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>232.08 ± 1.82</td>
<td>228.35 ± 0.38</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>155.40 ± 0.18</td>
<td>157.09 ± 0.36</td>
<td></td>
</tr>
<tr>
<td>T – test value</td>
<td>3.30</td>
<td>1.013</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Salmonella can both colonize and cause infections in humans and animals. Of interest, some Salmonella species appear to be better adapted to humans, and vice versa. For example, S.typhi does not have an animal reservoir and is solely transmitted by humans. Most human cases of Salmonella involve ingestion of a contaminated food item, in particular, eggs, poultry, ground beef, or dairy products (25). Salmonella can be acquired by direct personal contact, nosocomial transmission, or contaminated drugs/solutions (26). The result indicated anti-salmonella IgG was 40 (37%) of cases while IgM was 54 (50.9%) cases by immunochromatography method (Table 1).

Generally, the prevalence of infection is related to several factors including nutritional habits (27). The level of anti-PR3, in the serum of patients with Salmonellosis shows a significant increasing (P< 0.05) in comparison to healthy control, while the results shows no significant difference (P> 0.05) between the gender in both groups (Table 2). There are many explanations for the presence of the antibodies in Salmonellosis, one of them is the disruptive effect of salmonella on polymorph nuclear PMN expose, change intracellular proteins and rendering them antigenic city and result in the production of antibodies for epitope of anti -PR3 [28], also the other explanation is the antibody produced as a response to amoebic antigen, this antigen is cross-react with PMNL cytoplasmic components like anti PR3 [29].

The level of anti-MPO shows a significant increasing (P<0.05) in serum of patients with Salmonella in comparison with healthy control, while the results shows no significant differences (P>0.05) between the gender in both groups (Table 3). The results are in line with other studies which proved that PMNL granulocytes have an important role in innate immunity and their programmed cell death & removal are effective for acute inflammation resolution. MPO which is a hem protein generally associated with killing bacteria and tissue injury which oxidative, this property is expressed in
neutrophils, MPO binds to neutrophil (29).

The level of ALP increased significantly (P<0.05) in patients sera with Salmonellosis in comparison with healthy control, while the results showed no-significant difference (P>0.05) between the genders in both groups. The increasing level of alkaline phosphatase in the serum may be as a result to damage of liver cells and other cells infected with bacteria and released of the enzymes to blood stream (28).

The activity of Lactate dehydrogenase increase significantly (P<0.05) in patients sera of Salmonellosis in comparison to healthy control. While the results show no-significant difference (P>0.05) between gender in both groups. (Table-5). The increasing in LDH enzyme level may be due to cellular death and leakage of the enzyme from the infected cells (30).

**Conclusion**

The result indicated that anti –salmonella IgM positive in 54 cases, anti- salmonella IgG positive in 40 cases and 12 positive cases with both IgM and IgG. The Level of c-ANCA and p-ANCA, Alkaline phosphatase and Lactic acid dehydrogenase increased significantly, with no statistical difference between the gender of patients sera in comparison with healthy control.

**Conflict of Interests:** The authors declare that they have no conflict of interest

**Source of Funding:** Self –funding

**Ethical Clearance:** The researchers already have ethical clearance from College of Medicine, University of Baghdad and Ministry of health, Iraq

**References**


Preparation and Characterization of Some Complexes with New (Azo-Schiff base) Ligand and Study of Complex as Anticancer

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Abstract

A new series of transition metal complexes of Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Hg(II), Au(III) and Pt(II) have been preparation and characterization using anew azo-Schiff base ligand derived from Schiff base (N¹,N²Z)-N¹,N²-bis(4-amino-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-ylidene)benzene-1,2-diamine with 5-methyl-imidazole. The structures of the new ligand azo-schiff base and their transition metal complexes are characterized using several techniques, including analysis (C.H.N), molar conductance, magnetic measurements, electronic spectral, IR spectral studies, ¹³CNMR, ¹HNMR and mass spectra. The data show that these complexes have composition of [MLCl₂] where M=Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II), [ML]Cl₃ where M= Au(III) and [ML]Cl₂ where M=Pt(II). The electronic spectral, and magnetic susceptibility data of the complexes suggest octahedral geometry of all complexes, except the Au(III) and Pt(II) complex suggest a square planar geometry. The IR results demonstrate that the co-ordination sites are the azomethine nitrogen and azo nitrogen atoms of the ligand. The ligand behaves natural tetra dentate manner. The biological screening effect of the Pt(II) complex are tested against human breast cancer cell line MCF-7and normal cells (cell line WRL68). The results show the highest inhibitory effect for complex.

Key word: transition metal, schiff base ligand, imidazole

Introduction

Imidazole are heterocyclic have gained great importance as display wide range of pharmacological properties in addition to their excellent donor abilities and chelating agent, Schiff base and azo-Schiff bases important for the preparation because this class of compounds possess active \((\pi–acidic)\) for this reason a number of these compounds were prepared. Imidazole derivatives are of high interest among the researchers and uses in different fields because of their biological applications including antifungal, antioxidant, antiprototzoal, antihypertensive, antibacterial, and anticancer. Azo dyes are highly colored and have been used for dyeing industry, electrochromism, nonlinear optical elements, printing system, and pigments for a long time. On the other hand, azo compounds represent (60-70%) from all azo dyes, while Schiff base have played important role as chelating ligands in the development of coordination chemistry and optical materials, in addition to complexes show biomedical application like antimicrobial, anticancer, and antioxidant activities. Azo-Schiff base ligands and their complexes having multifunctional group play an important role due to their complexing, catalytic properties. Recently, this paper reports the synthesis, characterization and biological activity study of Pt(II) complex as anticancer of the new azo-Schiff base ligand \((3z)-N-(2-(((3z)-1,5-dimethyl-4-((4-methyl-1H-imidazol-2-yl)diazenyl)-2-phenyl-1,2-dihydro-3H-pyrazol-3-ylidene)amino)\) and its metal complexes with selected metal ions.

Measurement: The electrothermal melting point model 9300 was used to measure the melting point of the ligand and its complexes. Elemental analyses were carried out by means of micro analytical unit of 1180 (C.H.N) elemental analyzer. Electronic spectra were recorded on Shimadzu spectrophotometer double beam model 1700 Uv-Vis spectrophotometer–FTIR spectra were recorded in KBr disc on FTIR Shimadzu spectrophotometer model 8400 in wave number(4000-400)cm⁻¹. ¹H-NMR
&$^{13}$C-NMR-spectra in (ppm) unit were operating in DMSO-d$_6$ as solvent using (Bruker-Ultra Shield 3000 MHz Switzerland). And Mass Spectra were recorded on AB Scieix-3200 QTRAP LC/MS/MS. (Mass range-m/z(5-2000)-quad mode and(50-1700)linear ion trap mode). Magnetic susceptibility were carried out on a balance magnetic (MSB-MKI) using faraday method. The diamagnetic corrections were made by Pascal’s constants.

Preparation of ligands:

Preparation of the Schiff base[12]:

(4.06 gm, 0.02 mol) from 4-amino antipyrine dissolved in ethanol (50 ml) and then mixed with (1.08 g, 0.01 mol) (o-phenylene diamine) dissolved in ethanol. Three drops from glacial acetic acid were added and the mixture was refluxed with stirring for 35 hrs. Schiff base ligand was isolated after the volume of the mixture was reduced to half by evaporation and precipitated product was collected by filtered off and dried over anhydrous CaCl$_2$. Yield: 88%; mp: (125-127) C$^\circ$.

Preparation of the new azo-Schiff base ligand:

The new azo-schiff base ligand is prepared by coupling reaction of diazonium salt with appropriate amount of (5-methyl—imidazole) as coupling component in alkaline solution. Adiazonium solution is prepared by dissolving (4.78 g, 0.01 mole) of (N$^1$Z,N$^2$Z)-N$^1$N$^2$-bis(4-amino-1,5-dimethyl-2-phenyl-1H- pyrazol-3(2H)-ylidine)benzene-1,2-diamine in 4 ml of concentrated hydrochloric acid and (25 ml) distilled water. To this mixture a solution of (1.4 g, 0.02 mole) of sodium nitrate in 15 ml of distilled water was added drop wise at (0-5) $^\circ$C, and left to stand (30 min). This diazonium solution was added drop wise to 5-methyl—imidazole (1.64 g, 0.02 mole) dissolved in (30 ml) of ethanol and (50 ml) of (2 N) sodium hydroxide at (0-5) $^\circ$C. The mixture was allowed to stand overnight. The precipitate was filtered off, washed with distilled water, and recrystallized twice from hot ethanol and then dried in oven at 40 $^\circ$C for 10 hours. m.p(224-226) $^\circ$C, yield (60%), scheme 1.

Scheme-1: preparation of Schiff base, (azo-Schiff base) ligands and Complexes.

Preparation of metal complexes:

The metal complexes were prepared by mixing of 30 ml ethanol solution of (CoCl$_2$.6H$_2$O, NiCl$_2$.6H$_2$O, CuCl$_2$.2H$_2$O, ZnCl$_2$, CdCl$_2$.2H$_2$O, HgCl$_2$.2H$_2$O, PtCl$_2$. NaAuCl$_4$.H$_2$O) with 30 ml ethanol solution of (azo-Schiff base) ligand in (1:1) (metal: ligand) ratio. The resulting mixture was refluxed for 2 hrs. The product was isolated after reduced of volume by evaporation. It was filtered off, washed with ethanol and dried under vacuum. The complexes obtained are listed in table 2.

**Method**

Cytotoxicity Assays

To determine the cytotoxic effect, the MTT cell viability assay was conducted on 96-well plates. Cell lines were seeded at (1×10$^3$ cells/well). After (24 hrs). or a confluent monolayer was achieved, cells were treated with tested compound. Cell viability was measured after 72 hrs of treatment by removing the medium, adding 28 µL of 2 mg/mL solution of (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) reagentMTT (and incubating the cells for 1.5 h at (37°C). After removing the MTT solution, the crystals remaining in
the wells were solubilized by the addition of (130µL) of DMSO (Dimethyl Sulphoxide) followed by (37°C) incubation for (15min) with shaking.\(^{(13,14)}\). The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated as the following equation: \[ \text{Percent Inhibition} = \frac{(X - Y)}{X} \times 100 \]

### Results and Discussion

All our complexes are Freely soluble in DMF, DMSO, Methanol and Ethanol. Also, They are stable in air. The metal complexes were characterized by elemental analysis, molar conductivities, magnetic susceptibility, IR, UV-Vis, Mass and \(^{13}\)C, \(^{1}H\), MNR spectra. The analytical data of the complexes are in agreement with the experimental data. The value reveals that the metal to ligand ratio is (1:1) and are presented in Table 2. The magnetic susceptibility of the chelate complexes at room temperature were consistent with octahedral geometry, except the Pt(II) and Au(III) complex suggest a square planar geometry around the central metal ion. Most of chelate complexes prepared in this work showed lower conductivity values of the complexes. This proves that complexes have non-electrolytic nature. Except the Pt(II) and Au(III) complex which showed higher conductivity values support the electrolytic nature of the metal complexes.

### Micro analysis:

The elemental analysis data of 1:1 [M:L] ratio complexes showed that the theoretical values are in a good agreement with the found data, as listed in Table 1. The purity of azo-schiff base ligand was tested by TLC technique and C.H.N analysis.

<table>
<thead>
<tr>
<th>Table 1: Physical properties and analysis of Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>compound</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>L3</td>
</tr>
<tr>
<td>[CoL3Cl2]</td>
</tr>
<tr>
<td>[NiL3Cl2]</td>
</tr>
<tr>
<td>[CuL3Cl2]</td>
</tr>
<tr>
<td>[AuL3]Cl3</td>
</tr>
<tr>
<td>[PtL3]Cl2</td>
</tr>
<tr>
<td>[ZnL3Cl2]</td>
</tr>
<tr>
<td>[CdL3Cl2]</td>
</tr>
<tr>
<td>[HgL3Cl2]</td>
</tr>
</tbody>
</table>
Infrared Spectra studies of the ligand and complexes:

The important infrared spectral bands for the synthesized ligand and its chelate complexes are given in table.2. The ligand contains four potential donor sites: (1,2) the azometheine nitrogen, and (3,4) the azo group nitrogen. The IR spectrum of the ligand shows characteristic bands at (1639 and 1454) cm⁻¹ due to the (C=N) and (N=N) functional groups respectively. (15) The (C=N) and (N=N) bands in the free ligand shift to (1627-1614) cm⁻¹ and (1436-1409) cm⁻¹, respectively for the complexes. The reduction in bond order, upon complexation, can be attributed to delocalization of metal electron density (t²g) to the -system of the ligand. These shifts confirm the coordination of the ligand via the nitrogen of azo methine and the azo groups to metal ions. (16) The absorption band in free ligand observed at 3423 cm⁻¹ attributed to the (NH) group. (17) This band remains unchanged in the spectra of their complexes. The absorption band in ligand azo-schiff observed at 1666 cm⁻¹ attributed to the (C=N) of the N₃ imidazole nitrogen. This band remains unchanged in the spectra of their complexes. (18) This suggests that the (C=N) group is not taking part in coordination. New bands are attributed to (M-N) vibrations appearance in all complexes at (447-412) cm⁻¹ respectively. (19) The stretching wave number due to (N-N) in the coordinated compound was slightly affected from (1047) cm⁻¹ which indicate the un sharing of this linkage of pyrazolone ring in coordination with metal ions. Representative example for their spectra is given in Fig1.

**Table 2: Characteristic IR absorption bands of the ligand and its complexes in cm⁻¹**

<table>
<thead>
<tr>
<th>Compound</th>
<th>(N-H)</th>
<th>(C=N)imidazole</th>
<th>(N=N)</th>
<th>(C=N)Schiff</th>
<th>(N-N)</th>
<th>(M-N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligand</td>
<td>3423</td>
<td>1666</td>
<td>1454</td>
<td>1639</td>
<td>1047</td>
<td>------</td>
</tr>
<tr>
<td>[Cu L Cl₂]</td>
<td>3421</td>
<td>1660</td>
<td>1419</td>
<td>1625</td>
<td>1047</td>
<td>412</td>
</tr>
<tr>
<td>[Co L Cl₂]</td>
<td>3419</td>
<td>1666</td>
<td>1421</td>
<td>1624</td>
<td>1045</td>
<td>445</td>
</tr>
<tr>
<td>[Ni L Cl₂]</td>
<td>3412</td>
<td>1666</td>
<td>1436</td>
<td>1625</td>
<td>1045</td>
<td>445</td>
</tr>
<tr>
<td>[PtL]Cl₂</td>
<td>3421</td>
<td>1664</td>
<td>1411</td>
<td>1631</td>
<td>1045</td>
<td>441</td>
</tr>
<tr>
<td>[Zn L Cl₂]</td>
<td>3414</td>
<td>1665</td>
<td>1413</td>
<td>1622</td>
<td>1045</td>
<td>443</td>
</tr>
<tr>
<td>[Cd L Cl₂]</td>
<td>3419</td>
<td>1663</td>
<td>1413</td>
<td>1625</td>
<td>1045</td>
<td>428</td>
</tr>
<tr>
<td>[Hg L Cl₂]</td>
<td>3423</td>
<td>1665</td>
<td>1409</td>
<td>1627</td>
<td>1047</td>
<td>412</td>
</tr>
<tr>
<td>[Au L]Cl₃</td>
<td>3423</td>
<td>1663</td>
<td>1412</td>
<td>1614</td>
<td>1047</td>
<td>447</td>
</tr>
</tbody>
</table>

**Mass spectra:**

The mass spectra of synthesized ligand and its Cu(II) complex are recorded at room temperature. The obtained peaks confirm the proposed formulae for the compounds. The mass spectrum of Ligand show the molecular ion peak at m/z 798.2(0.9%) to the molecular formula (Cu(C₃₆H₅₆N₁₄Cl₂)consistent with the molecular weight of the Cu(II) complex.

**¹HNMR spectra:**

The ¹HNMR spectrum of the ligand shows the following signals: phenyl multiples at (7.3-7.5) ppm, =C-CH₃ at 2.3 ppm, N-CH₃ 3.3 ppm, CH₃ imidazole ring at
There is no appreciable change in all other signals in these complexes.

**13CNMR spectra:**

The spectra of the azo-Schiff base ligand were measured at room temperature in [D$_6$]DMSO. The $^{13}$C NMR spectrum of the ligand displayed characteristic signals at 8, 13.2, 39.3, 154.2 and 136.2 ppm due to (C–CH$_3$ imidazole), (>C–CH$_3$), (>N–CH$_3$), (>C–N=N–) antipyrine ring and (>C=N imidazole ring) of the azo-Schiff base ligand, respectively.\(^{(23,24)}\). The peak at $\delta = 164.9$ ppm was due to azomethine carbon of the ligand.\(^{(23)}\). Moreover, the spectrum of the ligand showed peaks in the region of (120.1, 124.2, 127.6, 128.6, 129.01 and 144.2, 146.0 ppm) due to aromatic carbon atoms.

**Electronic Spectra:**

Electronic spectra provide the most detailed information about the electronic structure. The UV-Vis spectrum of the (azo-schiff base) ligand exhibits two charge transfer (CT) bands at (310 nm) (32285 cm$^{-1}$) and (370 nm) (27027 cm$^{-1}$) attributed to ($\pi \rightarrow \pi^*$) and ($n \rightarrow \pi^*$) transition within the (azo-schiff base) ligand. In the spectrum of the complexes, the CT band at (310 nm) remains as such, in agreement with the $\pi \rightarrow \pi^*$ transition of the (azo-schiff base) ligand. The band observed at (370 nm) in the spectrum of the free ligand is red-shifted to (529–546 nm) in complexes due to ligand to metal charge transfer (LMCT) transition\(^{(25)}\), suggesting an octahedral geometry around metal(II) in the complexes.\(^{(26)}\). The electronic transitions, magnetic properties and conductivity values of the ligand and its complexes are listed in Table 3.

**Magnetic measurements:**

The Co(II) complex has a magnetic moment of 5.01 B.M, which is in agreement with the reported value for octahedral Co(II) complexes.\(^{(27)}\). The Ni(II) complex shows a magnetic moment value of (2.9) within the range of (2.9, 3.3) B.M\(^{(28)}\), suggesting an octahedral environment. The Cu(II) complex shows a magnetic moment value of 1.71 B.M, and consistent with a distorted octahedral geometry.\(^{(29)}\). The Zn(II), Cd(II), Hg(II), Au(III), Pt(II) are diamagnetic and according to the empirical formulae of complexes, an octahedral geometry is proposed\(^{(30)}\) except the Au(III) and Pt(II) complex suggest a square planar geometry.\(^{(31)}\).

---

**Table 3: Electronic spectra, conductivity and magnetic moment of complexes**

<table>
<thead>
<tr>
<th>Comp.</th>
<th>Absorption Bonds(nm)</th>
<th>Absorption Bonds(cm$^{-1}$)</th>
<th>Transition</th>
<th>$\mu_{\text{eff}}$(B.M)</th>
<th>Conductivity S.mol$^{-1}$ cm$^2$</th>
<th>Geometry</th>
<th>Hybridization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligand</td>
<td>310</td>
<td>32285</td>
<td>$\pi \rightarrow \pi^*$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>370</td>
<td>27027</td>
<td>$n \rightarrow \pi^*$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Pt L)Cl$_2$</td>
<td>543</td>
<td>18416</td>
<td>M→L,CT</td>
<td>Dia</td>
<td>75.03</td>
<td>square-planar</td>
<td>dsp$^2$</td>
</tr>
<tr>
<td>(Co LCl$_2$)</td>
<td>540</td>
<td>18518</td>
<td>M→L,CT</td>
<td>5.01</td>
<td>6.14</td>
<td>Octahedral distorted</td>
<td>sp$^3$d$^2$</td>
</tr>
<tr>
<td>[Ni LCl$_2$]</td>
<td>539</td>
<td>18552</td>
<td>M→L,CT</td>
<td>2.9</td>
<td>7.16</td>
<td>Octahedral (regular)</td>
<td>sp$^3$d$^2$</td>
</tr>
<tr>
<td>[Cu LCl$_2$]</td>
<td>546</td>
<td>18315</td>
<td>M→L,CT</td>
<td>1.71</td>
<td>9.22</td>
<td>Octahedral distorted</td>
<td>sp$^3$d$^2$</td>
</tr>
<tr>
<td>(Au L)Cl$_3$</td>
<td>535</td>
<td>18691</td>
<td>M→L,CT</td>
<td>Dia</td>
<td>166.17</td>
<td>square-planar</td>
<td>dsp$^2$</td>
</tr>
</tbody>
</table>
Anticancer activity:

Evaluation of newly synthesized complex in cancer therapy are studied. The antitumor activities of the synthesized Pt(II) complex was tested against breast cancer cell line MCF-7 and normal cells (cell line WRL68). The results showed that the highest inhibitory effect was reported for complex give IC50 value (93) and (1193), respectively. The cell cytotoxic effect of tested Pt(II) complex was calculated. The optical density was measured with the micro plate reader to determine the number of viable cells and the Inhibition Rate was calculated as

\[
\text{Percent Inhibition} = \frac{\text{Rate without inhibitor} - \text{rate with inhibitor}}{\text{rate without inhibitor}} \times 100
\]

The (50%) inhibitory concentration (IC50), the concentration required to cause toxic effects in (50%) of intact cells, was estimated from graphic plots of the dose response curve for each concentration. Using Graph pad Prism 6. The anticancer activity of the synthesized Pt(II) complex was determined against an human breast cancer cell line MCF-7 and normal cells (cell line WRL68) using different concentrations was evaluated. The cytotoxicity of Pt(II) complex on normal cells (WRL68) were studied using MTT assay. The results indicate effect of toxicity was very low on normal cells (WRL68). The platinum complex exhibit good results and expressed as IC50 value (μg/ml). The results are shown in fig. (1).

<table>
<thead>
<tr>
<th>Complex</th>
<th>λ (nm)</th>
<th>ε (M⁻¹cm⁻¹)</th>
<th>M→L, CT</th>
<th>Octahedral (regular)</th>
<th>sp³d²</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Zn LCl₂)</td>
<td>530</td>
<td>18867</td>
<td>M→L, CT</td>
<td>10.23</td>
<td>Dia</td>
</tr>
<tr>
<td>(Cd LCl₂)</td>
<td>529</td>
<td>118903</td>
<td>M→L, CT</td>
<td>8.01</td>
<td>Dia</td>
</tr>
<tr>
<td>(Hg LCl₂)</td>
<td>532</td>
<td>18796</td>
<td>M→L, CT</td>
<td>6.33</td>
<td>Dia</td>
</tr>
</tbody>
</table>

Fig. 1: Curve with plotting of IC50 of: (a) human breast cancer cell line MCF-7 & (b) WRL68
Treating tumors or tumor metastases

Killing cancer cells because of DNA binding cannot account for a selective mechanism of cell recognition and of discriminating toxicity. Provided that tumor cells differ from their healthy counterparts for the activated genes rather than for DNA content (which is almost identical in terms of nucleotide content, e.g. the number of guanines and their sequence, in any cell of the body including the tumor mass), the question remains open why, besides the high sensitivity of testicular tumors that can be cured, other tumors towards which platinum drugs are applied in a number of combination chemotherapeutic regimens show[32].

Conductivity measurement:

Molar conductance (Am) measurements of the metal complexes table (3) carried out using DMSO as solvent at the concentration of 10^-3M in room temperature. All chelate complexes prepared in this work showed conductivity values ranged between (6.14-10.23) s.mol^-1.cm^2 that non-electrolyte and no conductive species[15] While the molar conductance of Au(III) and Pt(II) complex are (75.03-166.17) s.mol^-1.cm^2 indicating the electrolytic nature (1:3) and (1:2) electrolyte of this complexes furthermore the chloride ions are located outside the coordination sphere[16].

Conclusions

In this paper we have explored the synthesis and coordination chemistry of some monomeric complexes obtained from the reaction of the tetra dentate ligand with some metal ions as shown in Figure (2). The mode of bonding and overall structure of the complexes were determined through physico-chemical and spectroscopic methods. Complex formation study via molar ratio has been investigated and results were consistent
to those found in the solid complexes with a ratio of (M:L) as (1:1). The biological screening effect of the Pt(II) complex are tested against human breast cancer cell line MCF-7and WRL68, the results show the highest inhibitory effect for complex.

Conflict of Interest: There is no any Conflict of Interest

Ethical Clearance: Ethics committee refer that there is no plagiarism and there is no mistakes or wrong results in work.

Source of Funding: Self funding.

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The Omega-3 Fatty Acids can Significantly Increase the Height of Children Under Five with Stunting

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Abstract

Children with stunting are a global public health problem that has implications for impaired physical growth, which has an impact on increasing morbidity and mortality. This study aims to study the role of omega-3 fatty acid supplementation on anthropometric measures of toddlers with stunting, especially height. This type of research was an experimental randomized clinical trial design, in the form of pre-post test control group design. Subjects who participated in this study were toddlers aged 12-36 months, consisting of 12 children as a control group who received placebo, and 12 children as a treatment group who received omega-3 fatty acid supplementation for 2 months. The parameters observed was height. The collected data were analyzed using t-test. The results of the study showed that there was a difference in changes in the subject’s height in the treatment group compared to the control group (p-value = 0.009294). It was further concluded that supplementation of omega-3 fatty acids in children under five with stunting could significantly increase height.

Keywords: omega-3 fatty acids, stunting, children under five, height

Introduction

Children with stunting are a global public health problem, which can increase morbidity and mortality. However, the most serious problem that needs important attention is the inhibition of cognitive and motor development due to brain volume that does not reach optimal size. In this regard, Martorell & Zongrone states that failure to promote linear growth has serious consequences, both in the short and long term, both on health and the formation of quality human capital.

Semba et al. through metabolomics studies concluded that there is a relationship between stunting and low serum omega-3 fatty acids. This nutrient is essential for growth and development. The results of his study also explained that interventions to reduce stunting could be done by increasing food intake of omega-3 fatty acid sources.

Koren et al. reported that exposure to omega-3 fatty acids at an early age can accelerate growth and improve bone quality. It was also explained that omega-3 fatty acids can improve bone health through increased absorption of calcium in the intestine, and increase osteoblast activity and differentiation, reduce osteoclast activity and encourage mineral deposits needed for bone development. Since 1929, Burr & Burr asserted that omega-3 fatty acids EPA and DHA are essential for health, and deficiency of these nutrients in the diet can cause illness. Then it was mentioned that omega-3 fatty acids EPA and DHA were concentrated in nerve tissue, including the brain, retina and breast milk. In particular, DHA is very much a phospholipid compound in the brain and is an essential component for brain growth. Based on the explanation above, supplementation of omega-3 fatty acids is expected to increase linear growth or height in children under five with stunting.

Material and Method

This study was an experimental research, a clinical trial using a randomized controlled trial (RCT), with a specific design that was pre-post test control group design. The sample size was 24 children under five, who were recruited from data available at the “Sikumana” Community Health Center, Kupang; with inclusion criteria namely healthy (physical, mental, social and normal activities), age: 12-36 months, nutritional status:
stunting (height / age <2 standard deviations), and their parents agree with their children’s involvement in the study this. The sample was divided into 2 groups namely; 12 children as a control group who received placebo and 12 children as a treatment group who received omega-3 fatty acid supplementation. The allocation of children under five to the group is done by simple random sampling, using a lottery.

The dose of omega-3 fatty acids given to the treatment group was adjusted for the child’s weight ie; body weight >10-20 kg given 500 mg of omega-3 fatty acids and children with a weight of ≤10 kg were given 350 mg of omega-3 fatty acids. This supplementation was given every day, for 60 days by health cadres.

The height of a toddler was measured using microtoise which has been calibrated before. Height measurements were carried out by health cadres and monitored or assisted by researchers. Height of children aged less than 2 years plus 0.7 cm as a correction. Height measurements were carried out in both groups in the phase before giving treatment (pre test) and after giving treatment (post test). The collected data was numerical data so that it was presented in the form of mean ± SD(7), and then analyzed using an independent sample t-test, about the difference in height gain between the treatment and control groups. Prior to the t-test, all data were proven to be normally distributed based on the results of the Saphiro-Wilk test and had a homogeneous variance based on the Levene test results.

Findings

The results of height measurements in the pre-test and post-test phases in the treatment and control groups are presented in Table 1.

Table 1. The mean score of height in the pre-test and post-test phases in the treatment and control groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Height (cm)</th>
<th>Difference (Δ)</th>
<th>n</th>
<th>Pre test</th>
<th>Post test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>0.63 ± 0.29</td>
<td>78.25 ± 5.63</td>
<td>78.87 ± 5.81</td>
<td></td>
</tr>
<tr>
<td>Intervention</td>
<td>12</td>
<td>1.12 ± 0.52</td>
<td>73.78 ± 7.15</td>
<td>74.88 ± 7.13</td>
<td></td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td>0.009294</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Referring to Table 1, it is known that the mean score of height increase in the treatment group (1.12 cm) was higher than the mean score of height increase in the control group (0.63 cm). Based on the results of the t-test, the difference in height gain from the two groups was significant, as evidenced by the p-value of 0.009, so that it could be interpreted that giving omega-3 fatty acid supplementation was effective to increase the height of a toddler with stunting.

Discussion

Based on the results of the study it is clear that the height increase of the group of children under five who received omega-3 fatty acid supplementation was significantly greater than the group of children who did not get the nutrient.

This condition is in line with the results of Koren et al., (2014) research on female mouse model fat-1 mice by administering high concentrations of omega-3 fatty acids to increase bone growth during pregnancy and postnatal periods. It can be concluded that exposure to high concentrations of omega-3 fatty acids at an early age can accelerate bone growth through changes in growth plates, increased proliferation and differentiation of chondrocytes. Lau, et al., (2013) reported that giving sunflower oil or fish oil for 12 weeks to growing mice had an impact on increasing calcium through absorption in the small intestine. In investigating the role of these unsaturated fatty acids in bone development, it is known that EPA and DHA increase mineralization, and then stimulate the differentiation of MSC (mesenchymal stem cells) into osteoblasts. The addition of arachidonic acid derived from omega-6 fatty acids inhibits the differentiation process into osteoblasts compared to cells that are given DHA. Then, the reduced omega-6 / omega-3 ratio indirectly in the bone has an effect on increasing osteoblast activity, which initiates an increase in bone formation early in life. It was also explained that omega-3 fatty acids could improve bone health through increased intestinal calcium absorption, and increased osteoblast activity and differentiation, reduced osteoclast activity and promoted mineral deposits in bone development.

Koren et al. reported that exposure to omega-3 fatty acids at an early age can accelerate bone growth and improve bone quality. In his experiments using genetically modified fat-1 mice whose bodies were able to synthesize omega-6 fatty acids into omega-3s,
it was found that there was accelerated bone growth as evidenced by the increasing length of the tails of both male and female mice. This acceleration rate of growth is the result of increased thickness of the bone growth plate, along with the thickening of the proliferation, prehypertropic and hypertropic zones. Then, the influence of omega-3 fatty acids is explained through two mechanisms, namely: (a) omega-3 fatty acids stimulate osteoblast differentiation through increased expression of parathyroid hormones and insulin-like growth factor 1, thereby increasing bone formation; (b) omega-3 fatty acids inhibit osteoclastogenesis through reduced NF-κB expression and signal modulation from RANKL, thereby reducing bone resorption.\(^{(4)}\)

In addition, Kajarabille et al. explained that omega-3 fatty acids can regulate bone metabolism, including reducing the release of prostaglandin E2 (PGE2) which is an important factor in osteoclast differentiation, nuclear kappa-β ligand (RANKL) receptor activation. Furthermore, these omega-3 fatty acids can modulate a number of proinflammatory cytokines, increase IGF-1 production and improve bone calcium enhancement. Thus, these fatty acids can maintain bone health and reduce bone loss.\(^{(8)}\)

Gyurko & Van Dyke explained that RvE1 is a derivative of omega-3 fatty acids that are effective in preventing and restoring bone loss in inflammatory bone disease periodontitis. This experiment provides evidence of the action of RvE1 on bone. Aside from being an anti-inflammatory, RvE1 also works on bone cells and promotes bone maintenance.\(^{(9)}\) Other evidence is shown by Gao et al that bioactive compounds derived from omega-3 fatty acids EPA and DHA can prevent bone loss and induce bone formation regeneration. It was also explained that RvE1 is a derivative of EPA omega-3 fatty acids which have anti-inflammatory and proresovin action mediated by the ChemR23 receptor which directly also functions to maintain bone through osteoblasts. In transgenic mice with ChemR23 receptors found additional anti-inflammatory action of RvE1 which directly impacts bone remodeling by suppressing bone resorption.\(^{(10)}\) The same thing was reported by Harerra et al. that RvE1 can inhibit osteoclast growth and bone resorption.\(^{(11)}\)

**Conclusion**

Based on the results of the study concluded that omega-3 fatty acids can significantly increase the height of children under five with stunting.

**Ethical Clearance:** This study had received ethical approval from the Health Research Ethics Commission, Faculty of Public Health, Universitas Airlangga, with number 322-KPK.

**Source of Funding:** Authors

**Conflict of Interest:** No

**References**

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Relations Exposure to Dust Inhalation Against Impaired Lung Function in Worker Unloading at the Port of Manado, North Sulawesi

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Abstract
Upper respiratory tract infection has been one of the top ten illness the last three years. In 2014 (28,579 cases), in 2015 (39,110 cases) and in August 2016 (27,068 cases). In the community health service of the Manado Port area, the upper respiratory tract infection is on the top list. The problem of loading and unloading is the problem of loading and unloading workers in Manado Port. The problem of loading and unloading workers in Manado Port. The research was observational with analytical approach and cross sectional design, with 60 samples of respondents. It consists of 9 variables and data was collected by interview, observation and measurement. (P = 0.195; PR = 0.648 (95% CI = 0.533 - 0.789). Smoking habit (p = 0.786; PR = 0.788 (95% CI = 0.361 - 1.719) Working period (p = 0.143; PR = 1.959 (95% CI = 0.896 - 4.283). Working duration (p = 0.838; PR = 0.831 (95% CI = 0.390 - 1.771) With potential variables. Affecting lung function impairment of loading and unloading workers is use of mask (p = 0.195) and period of work (p = 0.143). There is no significant correlation between use of mask, smoking habit, period of work, working duration, inhaled dust exposure and lung function disorder.

Keywords: inhaled dust exposure, lung function disorder.

Introduction
Among the environmental pollution, air pollution in Indonesia is a major determinant lingkungan quality, especially in big cities. Transport accounted for 60-80% of the air pollution. Some types of contaminants that are considered harmful to health community for example: PM2.5 and PM10, namely ka rbon monoxide, nitrogen dioxide, sulfur dioxide, particulates, hydrocarbons, CFCs, lead and carbon dioxide. Odorless and poisonous gas. The impact of air pollutants depends on the type of pollutant. PM10 is all particles that have a diameter of 10 microns. Because of its diameter, particles can enter the airway. Larger particles get stuck in the upper respiratory tract. But smaller particles measuring below 2.5 microns can enter the pulmonary alveoli and cause serious problems, from asthma to Chronic Obstructive Pulmonary Disease.

Respirable dust is dust or particles that are small enough that can enter into the nose to the upper respiratory system and into the inner lungs. Particles that enter the inner lung or inner respiratory system in general cannot released by the body’s mechanism system naturally (cilia and mucus) then as a result the particles will stay forever in the lungs.

The broad surface of the lungs, which is only separated from the thin membrane of the circulatory system, theoretically results in a person easily attacked by the entry of foreign matter (dust) and bacteria that
According to the International Labor Organization (ILO), an abnormality that occurs due to accumulation of dust in the lungs that causes a tissue reaction to dust is known as pneumoconiosis. Symptoms of pneumoconiosis include long coughing, long phlegm, fatigue, shortness of breath sometimes accompanied by wheezing.

Based on ILO data in 2013, 30% to 50% of workers in developing countries suffer from pneumoconiosis. Every year there are 2.3 million people in the world dying from work, both due to work-related illnesses and accidents. This figure is dominated by occupational diseases, namely 2.02 million cases of death.

Data on the prevalence of pneumoconiosis vary in each country in the world. SWORD data in the UK in 1980-1988 showed cases of pneumoconiosis of 10%. In Canada, cases of pneumoconiosis in 1992-1993 were 10%. While data in South Africa in 1996-1999 was 61%. In China from 1949-2001 the number of cumulative pneumoconiosis cases reached 569,129 cases.

Manado Harbor North Sulawesi, which is one of the inter-island connecting ports to the Sangihe and Talaud islands also to Ternate, every year has experienced increased activity, both people, goods and commodities between islands. Circulation of materials, commodities and chemicals and other materials always uses the port area as a route of transit before being sent to users/users. In fact, many chemicals are found stored in warehouses/storage located in the port area for days.

Several studies related to the use of Personal Protective Equipment (PPE) were carried out by Herlita, in the furniture industry in Makassar, showing that not using personal protective equipment (masks) had a significant relationship with a decrease in lung capacity. From the results of these studies it is known, strong relationship between respondents who did not use personal protective equipment with lung capacity and contributed 54.6% to the decline in lung function.

In the last three years ARI disease in the city of Manado ranks the top in 10 prominent diseases. In 2014 (28,579 cases), 2015 (39,110 cases) and 2016 in August (27,068 cases).

Based on preliminary studies at the Wonasa City Manado Public Health Center where the Manado port is included in its working area, for Acute Respiratory Infections (ARI) is the top-most prominent disease in ten prominent diseases that exist. Based on identification and interviews with puskesmas officers that some people with ISPA are laborers or members of TKBM in Manado port.

Based on the background of these problems, the authors suspect that the strong relationship between inhaled dust exposure and pulmonary dysfunction in loading and unloading workers at the port of Manado.

Material and Method

This study was an observational analytic study using a cross sectional design. In cross sectional analytic studies studying the relationship between risk factors and disease (effects), measurement of independent variables and dependent variables is done once at the same time.

The population in this study were all laborers transporting goods/materials from warehouses to ships, which are members of the Koperasi Sejahtera TKBM Manado City, which is 151 people. These transport workers are people who work exposed to dust in goods storage warehouses in the form of cement, fertilizers, pesticides, chemicals, bran, rice, and other basic commodities. The sample in this study is a portion of the population that is considered representative taken by using purposive random sampling technique and the determination of the sample size is calculated based on the Lemeshow formula. After calculated based on the formula, the number of samples was 60 respondents (minimum sample). The sample collection technique is purposive sampling.

Data collected directly by researchers against respondents. Data obtained are:

1. Measurement of inhaled dust levels using a PDS (Personal Dust Sampler)
2. Measurement of lung function capacity with a Spirometer tool
3. Measurement of body weight and height of respondents
4. Interview with respondents by filling out the questionnaire directly prepared (working period, length of work, mask use, smoking habits, exercise habits).

The data collected in this study was analyzed by
Chi-Square at a significance level of 5%.

Findings

The average age of respondents was 38.40 years standard deviation of 10.329. The minimum age of the respondent is 19 years and the maximum value is 58 years. The average working period of respondents is 9.10 years with a standard deviation of 7.489. The minimum value of the respondent’s tenure is 2 years and the maximum score is 38 years. The average length of work of the respondent is 8.60 hours with a standard deviation of 827. The respondent’s minimum length of work is 8 hours and the maximum value is 12 hours. Distribution of mask use is 54 (90%) using masks and 6 (10%) respondents who do not use masks. The distribution of smoking habits is 44 (73.3%) respondents who smoke and 16 (26.7%) who do not smoke.

Respondents with pulmonary disease disorders were experiencing 19 disorders (31.7%) and no interference namely 41 (68.3%). The average level of inhaled dust is 0.22 mg/m³ with a standard deviation of 0.322. The minimum value of inhaled dust is 0.28 mg/m³ and the maximum value is 1.028 mg/m³. The average lung capacity of respondents is 91.52% with a standard deviation of 21.214. The minimum value of the respondent’s lung capacity is 40% and the maximum value is 130%.

Risk Factors for Lung Function Disorders

The relationship between the use of masks and pulmonary dysfunction in loading and unloading workers at the Port of Manado.

In the variable use masks are grouped into not using masks and using masks. Data shows that of 54 respondents who did not use masks 19 (35.2%) respondents experienced pulmonary function disorders and 35 (64.8%) respondents did not experience pulmonary function disorders while those without pulmonary function, namely 16 (57.1%) respondents. Respondents who worked <10 years were 32 (100%) respondents, who underwent pulmonary function disorder, namely 7 (21.9%) respondents and 25 who did not experience pulmonary function disorders (78.1%).

The results of the bivariate analysis for the relationship between smoking habits and pulmonary dysfunction in loading and unloading workers at the Port of Manado obtained a significance value of 0.786, thus the probability (significance) was more than 0.05 (0.786 > 0.05), Ha was rejected or there was no significant relationship between smoking habits and pulmonary function disorders.

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The value of $p = 0.195$ is more than the significance of 0.05, so there is no significant relationship between smoking habits and pulmonary function disorders.

Risk Factors for Lung Function Disorders Work

Relationship of Work Period with pulmonary function disorders

tenure variables are grouped into 2 categories, namely work period> 10 years and 10 years totaling 28 (100%) respondents and 12 (42.9%) respondents who experience pulmonary dysfunction while those without pulmonary function, namely 16 (57.1%) respondents. Respondents who worked <10 years were 32 (100%) respondents, who underwent pulmonary function disorder, namely 7 (21.9%) respondents and 25 who did not experience pulmonary function disorders (78.1%).

The value of $p = 0.143$ is more than the significance of 0.05, so there is no significant relationship between years of work with pulmonary function disorders.

The results of the bivariate analysis for the relationship of working period with pulmonary dysfunction in loading and unloading workers at the Port of Manado, obtained a significance value of 0.143 thus the probability (significance) was more than 0.05 (0.143 > 0.05) then Ha was rejected or there was no meaningful relationship between work periods with lung function disorders workers unloading at the port of Manado.
Workers who are in a high dust environment for a long time have a high risk of pulmonary function disorders. According to Suma’mur, one of the potential variables that can cause lung function disorders is the length of time someone is exposed to these pollutants. This means that the longer the working period of a person, the longer the time of exposure to these pollutants.

**Long work relationship with pulmonary function disorders**

Long-term relationship variables are divided into 2 categories, namely working time > 8 hours and ≤ 8 hours. In the total data of respondents working > 8 hours as many as 28 respondents, 8 (28.6%) respondents experienced pulmonary function disorders and 20 (71.4%) respondents did not experience pulmonary function disorders. While respondents who worked ≤ 8 hours were 32 respondents, 11 (34%) had pulmonary function disorders and 21 (65.6%) did not experience pulmonary function disorders. The value of p = 0.838 is more than the significance of 0.05, so there is no significant relationship between the length of work with pulmonary function disorders.

The results of the bivariate analysis for the length of work relationship with lung function disorders of loading and unloading workers in the port of Manado, obtained a significance value of 0.838, thus the probability (significance) was more than 0.05 (0.838 > 0.05) then Ha was rejected or there was no meaningful relationship between the length of work and pulmonary dysfunction workers loading and unloading at the port of Manado.

**Relationship to inhalation of dust exposure with pulmonary function disorders**

Variable relationships inhaled dust exposure are divided into 2 categories, namely not fulfilling the requirements and fulfilling the requirements. In the data shows that 60 respondents or total respondents there were no respondents who experienced exposure to inhaled dust that did not meet the requirements. Respondents with exposure to inhaled dust who fulfilled the requirements were 19 (31.7%) respondents experiencing pulmonary function disorders while 41 (68.3%) respondents did not experience pulmonary function disorders.

The results of data analysis for respondents exposed to inhaled dust below the Threshold Value (NAB) amounted to 60 (100%) respondents. There were no respondents exposed to dust above the NAB. In line with the research conducted by Fordiastiko, there were no significant abnormalities of pulmonary physiology between groups of cement factory workers who worked with dust levels above the NAB with those below the NAB (p = 0.509). The size of dust or particles that enter into the lung will determine the location of attachment or deposition. Human sucked particles less than 1 micron in size will come out when exhaled breath. Particles measuring 1-3 microns will enter the air bag of the lungs, attaching to the alveoli.

**Conclusion**

There was no significant relationship between mask use (p = 0.195), smoking habits (p = 0.766), work period (p = 0.143), length of work (p = 0.838) and exposure to inhaled dust (100% below NAB) with pulmonary dysfunction in loading and unloading workers at Manado Harbor. The most potential variable associated with pulmonary function disorders in loading and unloading workers in the Port of Manado is the use of masks with a value of p = 0.195 and years of service with a p value = 0.143.

**Conflicts of Interest:** All authors have no conflicts of interest to declare.

**Source of Funding:** The source of this research costs from self.

**Ethical Clearance:** This research was approved by the Institutional Ethics Board of Airlangga Surabaya University. All subjects received complete information about the purpose of this study, each subject before the study signed an informed consent form.

**References**


Urinary Tumor Markers for Detection and Surveillance in Non-Muscle Invasive Bladder Cancer

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Abstract

Objectives: Cystoscopy still remains the gold standard for diagnosis and follow-up monitoring of bladder cancer (BC). Unfortunately, it is an invasive, and the urine cytology is a highly specific, sensitivity for detection of low grade tumors is only 4% to 31%. The advent of noninvasive urine-based markers as well as other novel modalities has yielded improved diagnostic accuracy.

Material and method: Pubmed/Medline search was conducted to identify original articles, review articles, and editorials regarding urine-based biomarkers for screening, early detection, and surveillance of urothelial carcinoma of the bladder. Results: urinary marker shave shown higher sensitivity compared with cytology, and most markers suffer from lower specificity than cytology. In this review, we aimed to summarize the current knowledge on commercially available and promising investigational Urinary tumor markers for detection and surveillance in Non-Muscle Invasive BC. Conclusions: biomarkers into clinical decision making will be of value for BC detection and screening in the future.

Keywords: Bladder cancer (BC), Biomarkers, Urinary marker.

Introduction

Bladder cancer (BC), a highly aggressive and heterogeneous disease, is the most common malignancy of the urinary tract. (1). The highest incidence rates of BC are generally found in industrially developed Countries².

Early diagnosis of BC allows for effective local treatment and optimizes the success of surgical therapy.(3). In this review, we use this framework to discuss the urinary biomarkers for early diagnosis of BC.

1. Cytology

Urinary cytology has been established as the standard noninvasive urinary marker for detecting BC. High-grade cancers are more likely than low-grade tumors to shed their cells into the urine due to their weaker intercellular attachments (1). Many studies demonstrated a poor sensitivity for low-grade BC (13% to 75%), with improved sensitivity and specificity for high-grade disease. The sensitivity for high-grade cancers has been over 80%. The overall sensitivity of cytology ranges from 25 to 70% (2).

2. ImmunoCyt

ImmunoCyt combines cytology with an immunofluorescence assay(3). It detects cellular biomarkers for BC in exfoliated urothelial cells using fluorescent monoclonal antibodies for a high molecular weight form of carcinoembryonic antigen and two bladder tumor cell associated mucins. The test requires trained personnel, it is expensive, and a large number of exfoliated cells are necessary to perform an accurate test(4). ImmunoCyt has a reported overall sensitivity of 50–100% (5). Its specificity has been reported as 69–79%, with a higher false positive rate in patients with benign prostatic hyperplasia or cystitis(4, 5).

3. Fluorescence in situ hybridization (FISH)

FISH is a molecular test that utilizes DNA probes to identify the most common urothelial carcinoma related chromosomal changes in exfoliated cells in the
The test was designed to detect aneuploidy of chromosomes 3, 7, 17 and the loss of the 9p21 locus in malignant urothelial cells (6).

The sensitivity of the UroVysion FISH test to be between 69 and 85%, with a specificity of 78 to 92% (7, 8). In a direct comparison of FISH with cytology and NMP-22, FISH was found to have better sensitivity for low grade tumors. The combination of FISH and cytology detected 97.4% of cancers, while the combination of cytology and NMP-22 detected 92.1% (9).

4. Aurora kinase A (AURKA)

The Aurora kinase A (AURKA) gene encodes a serine/threonine kinase associated with aneuploidy and chromosome instability. This gene has been explored in urine sediment by FISH. A training set was used to establish test conditions. A separate testing set of 100 patients with BC, 92 healthy individuals, and 56 patients with benign urologic disease reported a test sensitivity of 87% and a specificity of 97% (10).

5. Cytokeratins

Cytokeratins are intermediate filaments proteins that are part of the framework of eukaryotic cells; their main function is to enable cells to withstand mechanical stress. In humans 20 different cytokeratin isotypes have been identified. Cytokeratins 8, 18, 19 and 20 have been associated with BC (11). The urinary BC test detects cytokeratin 8 and 18 fragments in urine. The sensitivity of the urinary BC test varies from 35% to 79% (12).

6. Bladder tumor antigen (BTA)

BTA stat and BTA-TRAK are designed to detect bladder tumor associated antigen in voided urine. This antigen is a human complement factor H–related protein similar to human complement factor H. BTA interacting with complement factor C3b interrupts the complement cascade, potentially conferring a selective growth advantage to cancer cells. In cell culture, normal cells do not express the H-related protein (13).

The sensitivity and specificity of BTA stat ranged from 67 to 70% and 75 to 78%, respectively. The sensitivity and specificity of BTA-TRAK was noted at 66 and 65%, respectively (14). BTA stat demonstrates improved sensitivity with increasing histological grade; 53, 76, and 90% for grades 1 through 3. It has also been noted to have improved detection and sensitivity for superficial and low-grade tumors compared to urinary cytology; however, at the cost of lower specificity (13). The infection or urinary lithiasis, may lead to a false positive for BTA. In addition, false positives may be seen in patients with a history of Bacillus Calmette-Guerin (BCG) therapy or urinary diversions utilizing the bowel.

7. LEWIS X

Monoclonal antibodies have also been utilized to detect the Lewis X antigen on exfoliated urothelial cells. The Lewis X is a blood group antigen that is normally absent from urothelial cells in the adult, but is expressed in transitional cell tumors regardless of the secretor status, grade, or stage of the tumor (15). Commercially available antibodies, such as the P-12 murine monoclonal antibody, have been utilized to target and detect the Lewis X antigen (8).

8. ACCU-DX

The Accu-Dx test was developed as a qualitative point-of-care immunoassay utilizing murine monoclonal antibodies specific for (fibrin/fibrinogen degradation products) FDP. However, since these antibodies have also been noted to react with intact fibrinogen typically found in human serum, the usefulness of the test in the presence of hematuria may be low (16).

9. Hyaluronic acid (HA)

HA is an extracellular glycosaminoglycan that supports tumor cell adhesion and migration, and offers some protection from immune system surveillance in tumor tissues. Small fragments of HA stimulate angiogenesis and are produced by hyaluronidase (HAase). The small HA fragments have been identified in the urine of patients with BC (16). The importance of HA and HAase in BC pathogenesis has been noted in a study in which staining of BC tissues for HA and HAase correlated with muscle-invasive disease and recurrence (17). Hyaluronoglucosaminidase-1 (HYAL-1) is a specific HAase that has been identified as a marker for cancer detection, and is a molecular predictor for tumor growth, invasion, and angiogenesis. HYAL-1 mRNA levels have been noted to be elevated 10 to 30 fold higher in bladder cells and tissues that express high HAase activity, as well as in the urine of patients with BC (16).

10. Microsatellite Analysis

One of the most common genetic changes in BC is
loss of heterogeneity in chromosome 9. Chromosomes 4p, 8p, 9p, 11p, and 17p also often display loss of heterogeneity in patients with BC(18).

Microsatellite analysis evaluates for tumor specific genomic alterations in a different manner than FISH. Microsatellite analysis targets highly polymorphic, short tandem repeats. This technique evaluates the shift in the normal ratio of two alleles that occurs with genomic alterations from tumor cell transformation. This loss of heterozygosity can be used as a biomarker of a suspected neoplastic process. It had already been established that microsatellite changes in urine samples matched DNA extracts from tumor tissues(19).

11. BLCA-1 and BLCA-4

BLCA-1 and BLCA-4 are nuclear transcription factors present in BC. BLCA-1 is not expressed in nonmalignant urothelium, whereas BLCA-4 is expressed in both the tumor and adjacent benign areas of the bladder but not in nonmalignant bladders(20).

A nuclear matrix protein specific to BC tissues, termed BLCA-4, has been identified. It holds great promise as a potent urine based bladder tumor marker(5).

12. CEACAM1

Bladder tumor growth and progression depend on angiogenesis. Human carcinoembryonic antigen-related cell adhesion molecule (CEACAM)1 is a cell adhesion molecule with proangiogenic activity. It has previously been observed that CEACAM1, which is ubiquitously expressed in the luminal surface of normal bladder urothelium, is down regulated in BC cells while it is concurrently up regulated in endothelial cells of adjacent blood vesse (5). This differential switch in CEACAM1 expression is accompanied by an up regulation of proangiogenic and prolymphangiogenic factors. Based on these findings, it was assessed whether CEACAM1 was detectable in urine and whether its levels could help differentiate BC patients from healthy subjects(5).

13. MicroRNA markers

MicroRNAs (miRNAs) are noncoding RNAs that post transcriptionally regulate gene expression(21). They might serve as an ideal bladder biomarker because they are stable within urine and require little handling care(22), and they are more stable against nuclease degradation due to their small size. Urine contains many nucleases, and assays to examine mRNA expression often fail due
to target degradation or require stringent prelaboratory handling of the urine sample. Recently, urinary miRNA expression was reported and the upregulation of miRs-126/182/199a was found to discriminate BC patients from disease-free controls(23).

14. Survivin

Survivin, a novel member of the inhibitor-of-apoptosis gene family, is prominently reexpressed in many types of cancer. Survivin messenger ribonucleic acid (mRNA) is overexpressed in human cancers and can be detected in urine using a bio-dot immunoassay incorporating a rabbit polyclonal antisurvivin antibody(24). Urinary levels of survivin gene activation, both at the protein and the mRNA level are associated with BC presence, higher grade, and advanced pathologic stage(5).

15. Proteomics and genomics

Advances in the fields of molecular genetics and tissue microarrays have led to the development of many potential novel biomarkers. The chemokine CXCL-1 and the matrix metalloproteinases MMP-2 and 9 have been identified using these immunochemical techniques as potential urine based markers(25).

16. Additional urinary markers

Many other potential biomarkers have been evaluated for their potential usefulness as a urine-based tumor marker. Some of the more promising of these have included cytokeratin CK20, The CK20 assay has 78 to 87% sensitivity and a 56 to 80% specificity for BC detection. A summary of these current and emerging bladder tumor biomarkers are shown in, Table (1)(8).

17. Nuclear matrix proteins (NMP)

NMP-22 is a nuclear mitotic apparatus that is involved in proper distribution of chromatin to daughter cells during cellular replication(25). It is located in the mitotic spindle during mitosis. NMP-52 and NMP-22 is present at a relatively low level in the interphase nuclear matrix. It is probably released from the nuclei of the tumor cells during apoptosis. NMP-52 and NMP-22 concentration is at least 25 fold greater in BC than in mean levels isolated from normal bladder and also five times greater in urine from patients with pyuria, urolithiasis, hematuria, and cystitis comparing with normal bladder(1, 26).
The NMP marker was identified in the urine of patients with BC at 52 kDa. The dot-ELISA detected the urinary NMP-52 marker in 92% of patients with squamous cell carcinoma, 98% with transitional cell carcinoma, and all six of those with adenocarcinoma of the bladder, with a specificity of 94% (27).

Table (1). Summary of urinary markers and their performance(8).

<table>
<thead>
<tr>
<th>Urinary Marker</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>Clinical Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytology</td>
<td>12.2-79</td>
<td>78.4-99.4</td>
<td>Laboratory</td>
</tr>
<tr>
<td>Quanticyt</td>
<td>42.1-69</td>
<td>67.9-87</td>
<td>Investigational</td>
</tr>
<tr>
<td>FISH</td>
<td>69-92.1</td>
<td>89-94.5</td>
<td>Laboratory</td>
</tr>
<tr>
<td>NMP-22</td>
<td>49.5-92.1</td>
<td>66-87.3</td>
<td>Laboratory &amp; point of care</td>
</tr>
<tr>
<td>BAT-Stat</td>
<td>50-70</td>
<td>67-78</td>
<td>Point of care</td>
</tr>
<tr>
<td>Immunocyt</td>
<td>66.7-84.9</td>
<td>62-84.7</td>
<td>Laboratory</td>
</tr>
<tr>
<td>FDP (Accu-Dx)</td>
<td>52-68.4</td>
<td>79.6-91</td>
<td>Point of care</td>
</tr>
</tbody>
</table>

Telomrase:

| TRAP                 | 77.4-90       | 88-93.5       | Investigational          |
| Htert                | 84.8-95       | 43.8-93.5     |                          |

Hyaluronic acid:

| HA                   | 61-83.1       | 53.6-90.1     | Investigational          |
| HYAL-1               | 57.6-91       | 78-100        |                          |
| HAase                | 81.5          | 83.8          |                          |
| HA/HAase             | 88.1-94       | 63-84.4       |                          |
| Lewis X              | 79.8-84       | 80-86.4       | Investigational          |
| Survivin             | 75            | 100           | Investigational          |
| LOH                  | 60-97         | 93            | Investigational          |
| BLCA-4               | 89-94.4       | 95-100        | Investigational          |
| UPK3A                | 83            | 83            | Investigational          |

Discussion

Urinary cytology is lacking the sensitivity for different grades of BC specifically in low-grade tumors (1, 2). Urine markers have been investigated; however, to date, no marker has reached widespread use. Although urinary markers have shown higher sensitivity compared with cytology, most suffer from low specificity (18). Combination of different markers is promising concept and seems to represent the future. Another limitation is that each setting (screening/early detection and surveillance) suffers from different requirements. Finally,
each marker has also to prove its cost effectiveness and also the advent of noninvasive urine based markers as well as other novel modalities has yielded improved diagnostic accuracy (26).

Conclusions

Future studies are needed to clarify the prospective biological markers in different stage and grade of BC; The combination of urinary markers and urine cytology is a good diagnostic tool for BC in different pathological types of BC.

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Conflict of Interest: None

Ethical clearance: The study protocol conformed to the ethical guidelines of the 1975 Helsinki Declaration and the approval was obtained from ethical committee of Middle East University-Amman-Jordan.

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References


Relationship between QT Interval Dispersion and Degree of Coronary Artery Disease

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Abstract

Background: It has been shown that QT dispersion (QTD) increases during episodes. Current study has been done in order to determine QTD in patients with stable angina, assess myocardial ischemia and infarction.

Aims of Study: This study aiming to determine the relationship between severity of coronary artery disease (CAD) and degree of QTD.

Patients and Method: A 214 patients (177 men and 37 women) underwent diagnostic coronary angiography because of suspected CAD. Standard resting 12 lead electrocardiogram (ECG) were recorded within 24hrs before coronary angiography. QT intervals were measured manually by ruler method and QTD were gauged as (QT maximum – QT minimum).

A 184 patients had CAD and control group (30 persons) had a normal coronary angiogram

Interestingly, QTD has increased significantly as there was sever increment of CAD. QTD was in 1 vessel disease, 2 vessel disease, in 3 vessel disease, and in left main stem disease.

Left ventricular dysfunction has raised QTD significantly in patients with 1,2 vessels, as well as left main stem disease.

QTD clearly elevated as Gensini score increased. Involvement of proximal left anterior descending artery did not increase QTD significantly in patients with CAD.

Multiple regression analysis demonstrated that severity of CAD, left ventricular dysfunction and previous myocardial infarction were independently associated with increased QTD.

QTD increased significantly in patients with stable angina compared to the controls. Severity increase of CAD resulted in significant increase in QTD, and presence of LV dysfunction has caused further increase.

Introduction

QT dispersion (QTD) has been defined as difference between maximum and minimum QT intervals in any number of Electrocardiography (ECG) leads [1, 2] and related to electrical instability and risk of ventricular arrhythmogenesis [2].

QTD increased in patients with coronary artery disease (CAD) [3-5] and it especially prolonged during active ischemia [6, 7].

In present study we tried to determine QTD in patients with chronic stable angina as measured by surface ECG at rest. As well as, to assess the relationship between severity of CAD as assessed by coronary angiography and the degree of QTD.


Patients and Method

Between April 2001-2002, 2106 patients underwent cardiac catheterization and coronary angiography for definite or suspected Ischemic Heart Disease (IHD) in Ibn Albitar Hospital for Cardiac Surgery. Of those patients 214 (177 men and 37 women) fulfilled the following criteria.

1. Coronary angiography because of suspected CAD.
2. No valvular disease.
3. No sign of LV hypertrophy on LV angiogram or Echo study.
4. No evidence of unstable angina or acute myocardial infarction (MI).
5. ECG: sinus rhythm, no bundle branch block, with a well-defined T wave in all or most (10 or more of the 12 leads ECG) leads that make accurate measurement of QT interval possible.
6. Normal levels of serum potassium and calcium.
7. No medication, known to induce prolongation or shortening of QT interval or change QTD.

Detailed history and careful physical examination have been applied for all patients.

Standard resting 12-lead ECG with simultaneous lead acquisition were recorded with Mac 500, version 2.2 (Marquette Hellige GmbH, Freiburg, Germany) at speed 25 mm/second.

Diagnostic coronary angiography and left ventriculography were done within 24 hours of the ECG recording.

Coronary Angiography

Left heart catheterization was done using Judkin’s technique (percutaneous transfemoral) [9]. Coronary angiography has been done in multiple views. Coronary artery lesions were considered significant in cases when stenosis was at least 70% (or 50% left main coronary artery) [10].

Furthermore, coronary angiography was evaluated as single, double or triple vessel disease [8, 11] by quantification of the lesions according to Gensiniscore [12].

In addition, left ventricular function was assessed by single plane left ventricular angiogram in the 300 right anterior oblique positions [13], as well as by two D and M modes Echocardiography [14]. Moreover, normal LV function was defined as ejection fraction of 55% or more [15].

Mild LV dysfunction was defined as ejection fraction (EF) between 40-55%. Finally, moderate LV dysfunction 30-40% and severe LV dysfunction < 30% [15].

Analysis of ECG

Einthoven lead II [8] was analyzed in order to measure QT and corrected QT (QTc) intervals.

QT intervals were measured manually (ruler method) [16] in all limb and chest leads by two observers and were measured from the onset of QRS complex (from the first deflection of QRS complex) to the point of return of the T wave to the isoelectric line [3, 8, 17]. More specifically, when a U wave followed the T wave, the end of the T wave was taken as the nadir between the T and U waves [6]. In case that the end of the T wave could not be identified assuredly, that lead was excluded from the analysis.

Notably, the QT interval was measured in three consecutive complexes in each lead and the average value was relied [6].

QTD was calculated as the difference in ms between longest and shortest measured QT intervals [3, 6, 16 & 18].

No attempt was made to correct for missing leads.

For the measurement of QTc interval of lead II modified Bazett’s formula was applied QTc=QT/RR [1, 8].

Patients were considered normal in term of coronary arteries when there was no angiographic stenosis, no clinical, as well as ECG or angiographic evidence of previous myocardial infarction [6].

Statistical Analysis

All data were given as mean ± standard deviation. Analysis of variance (ANOVA) was used for comparison among variances.

P ≤ 0.05 was considered significant.
Results

The study population was composed of 184 patients with CAD with a mean of age (54.75± 9.19 years) and 30 patients with normal coronary angiogram (control group) with a mean of age (Table 1).

No significant difference was found between the two groups in term of age; however hypertension, diabetes mellitus (D.M.) and smoking were more common among those with CAD. Interestingly, male: female ratio was significantly higher in CAD patients.

Among patients with CAD, ECG evidence of Q wave MI was present in 90 patients that represent (48.9%).

Patients with CAD have been classified according to the severity of their disease as single, two, three- vessel or left main stem (LMS) disease (Table 2). Moreover, those with more extensive disease tended to be older with most common hypertension and D.M. In contrast, there was no significant difference in relative to gender or smoking within the different groups of CAD patients.

No significant difference in the incidence of Q wave MI or LV dysfunction in all groups of CAD patients was detected. In addition, our results have illustrated that QTD significantly increased as severity of CAD elevated (Fig. 1). However, no significant difference in QTD was found between those with three vessels disease and LMS disease.

Patients with CAD (one, two, three vessels or LMS disease) are further stratified according to their LV function (Table 3). LV dysfunction induced QTD significantly in patients with one, two vessels and LMS disease.

We noticed that the occurrence of Q wave MI among those with LV dysfunction was significantly higher than those with normal LV function in patients with one, two vessels and LMS disease.

If Gensini score is used to assess the severity of CAD (Fig. 2), so the QTD is clearly raised as the score increased.

We also found that there was no significant difference in QTc interval when compare normal subjects to those with CAD, or among different groups of patients with CAD (Table 3).

In the current study, when patients with CAD are stratified according to LV function and involvement of proximal left anterior descending (LAD) artery (Table 4).

Table 1. Characteristics of study population.

<table>
<thead>
<tr>
<th>Number</th>
<th>Control group</th>
<th>IHD patients</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>51.5±11.2</td>
<td>54.75±9.19</td>
<td>NS</td>
</tr>
<tr>
<td>Male gender (%)</td>
<td>19(63.2%)</td>
<td>158(85.87%)</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>12(40%)</td>
<td>108(58.69%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>D.M(%)</td>
<td>1(3.3%)</td>
<td>66(35.87%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>8(26.7)</td>
<td>109(59.245)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
Fig 1: QTD in healthy individuals and in patients with CAD

![Graph showing QTD in healthy individuals and in patients with CAD](image)

Fig 2 QTD data for patients classified using Gensini Score

Table 2. Characteristics of patients with IHD.

<table>
<thead>
<tr>
<th></th>
<th>1 vessel</th>
<th>2 vessels</th>
<th>3 vessels</th>
<th>LMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>56</td>
<td>60</td>
<td>30</td>
<td>32</td>
</tr>
<tr>
<td>Age (years)</td>
<td>50.05±8.91</td>
<td>54.82±7.76</td>
<td>56.7±9.0</td>
<td>61±8.47</td>
</tr>
<tr>
<td>Male gender (%)</td>
<td>44(78.57)PNS</td>
<td>60(90.91)PNS</td>
<td>24(80)PNS</td>
<td>30(92.72)</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>30(53.33)PNS</td>
<td>35(53.03)*</td>
<td><strong>33(76.67)</strong></td>
<td><strong>20(62.5)</strong></td>
</tr>
<tr>
<td>D.M.(%)</td>
<td>14(25)PNS</td>
<td>20(30.3)*</td>
<td><strong>16(53.33)PNS</strong></td>
<td><strong>16(50)</strong></td>
</tr>
<tr>
<td>Smoking(%)</td>
<td>29(31.79)PNS</td>
<td>42(63.64)PNS</td>
<td>14(46.67)*</td>
<td>12(44.75)</td>
</tr>
<tr>
<td>Q-wave MI(%)</td>
<td>28(50)PNS</td>
<td>30(45.45)</td>
<td>14(46.67)PNS</td>
<td>18(56.25)</td>
</tr>
<tr>
<td>LV dysfunction(%)</td>
<td>14(25)PNS</td>
<td>18(27.3)PNS</td>
<td>2(6.7)*</td>
<td>8(25)</td>
</tr>
</tbody>
</table>
Table 3. ECG variables of control group and patients with CAD.

<table>
<thead>
<tr>
<th></th>
<th>LVEF &gt; 50</th>
<th>LVEF ≤ 50 F &gt; 50</th>
<th>LVEF ≤ 50 F ≤ 50</th>
<th>LVEF ≤ 50</th>
<th>LVEF &gt; 50</th>
<th>LVEF ≤ 50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>30</td>
<td>42</td>
<td>14</td>
<td>48</td>
<td>18</td>
<td>24</td>
</tr>
<tr>
<td>_Q-MI(%)</td>
<td>0</td>
<td>17 (40)*</td>
<td>11 (78.57)</td>
<td>19 (39.58)*</td>
<td>11 (61.6)</td>
<td>12 (42.86)</td>
</tr>
<tr>
<td>QTc</td>
<td>415.2?</td>
<td>495.93</td>
<td>414.28 ?</td>
<td>471.75</td>
<td>447.28 ?</td>
<td>411.78</td>
</tr>
<tr>
<td>±30.9</td>
<td>±33.35?</td>
<td>±52.11</td>
<td>±34.67 ?</td>
<td>±70.47</td>
<td>±49.26</td>
<td>±414.0</td>
</tr>
<tr>
<td>±QTD</td>
<td>±30.6?</td>
<td>65.62</td>
<td>84.57</td>
<td>77.1?</td>
<td>89.77</td>
<td>95.71</td>
</tr>
<tr>
<td>±16.39</td>
<td>±22.93*</td>
<td>±28.52</td>
<td>±20.61*</td>
<td>±29.26</td>
<td>±11.36%</td>
<td>±7.07</td>
</tr>
</tbody>
</table>

Table 4. QTD and QTc in patients with CAD classified according to LV function and involvement of proximal LAD artery.

<table>
<thead>
<tr>
<th></th>
<th>1 vessel</th>
<th>2 vessels</th>
<th>3 vessels</th>
<th>-LAD</th>
<th>LAD+LAD</th>
<th>LAD+LAD</th>
<th>-LAD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Good LV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>10</td>
<td>32</td>
<td>12</td>
<td>36</td>
<td>16</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>QTc</td>
<td>407.9</td>
<td>405.3</td>
<td>431.58</td>
<td>410.69</td>
<td>401.63</td>
<td>429.08</td>
<td></td>
</tr>
<tr>
<td>±20.56*</td>
<td>±36.74</td>
<td>±39.83*</td>
<td>±39.39</td>
<td>±57.31*</td>
<td>±36.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>QTD</td>
<td>74±20.65</td>
<td>63±23.28</td>
<td>81±14.35</td>
<td>75.7</td>
<td>95</td>
<td>96.67</td>
<td></td>
</tr>
<tr>
<td>±23.28*</td>
<td></td>
<td></td>
<td>±22.31</td>
<td>±8.94*</td>
<td>±14.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>LV dysfunction</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>8</td>
<td>6</td>
<td>2</td>
<td>16</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>QTc</td>
<td>388.75</td>
<td>448±51.93</td>
<td>329±1.41</td>
<td>462.06</td>
<td>414</td>
<td>±2.38</td>
<td></td>
</tr>
<tr>
<td>±37.89*</td>
<td></td>
<td></td>
<td>**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QTD</td>
<td>78.77</td>
<td>80.3±20.65</td>
<td>87±2.83</td>
<td>90.1</td>
<td>95±7.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>±31.51*</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P < 0.05
-LAD No proximal LAD critical lesion
+LAD Proximal LAD critical lesion
Discussion

Present study has appraised relationship between QTD and severity of CAD in patients with angina pectoris. Observations of our study have showed that hypertension, diabetes mellitus, smoking and male gender were more prevalent among patients with CAD in comparison to control group. This prevalence indicates the impact of these risk factors in increasing coronary atherosclerosis [11, 19].

Among patients with CAD, we found that those with more extensive disease tended to be older, with most common hypertension and diabetes mellitus. Previous study has revealed that prevalence and severity of coronary artery atherosclerosis increase dramatically with age advance [11]. Our interpretation is that may reflect the cumulative nature of atherogenesis [20]. In addition, it has been demonstrated that aging is associated with higher incidence of hypertension, diabetes mellitus, disturbed lipid profile and obesity [11].

We also found that QTD is significantly increased among patients with stable angina than healthy controls. In agreement with our observations, Tikiz et al [21] has showed that patients with single vessel disease had wider baseline QTD.

Numerous studies have evaluated QTD in patients with CAD during active ischemia either induced by atrial pacing [6], exercise test [23], spontaneous anginal episodes [23] or after acute MI [3].

Transient myocardial ischemia followed by reperfusion, or chronically ischemic and hypo perfused myocardium may result in increased production of superoxide and hydroxyl radicals [24], this leads to impaired sarcolemmal Na+, K+-ATPase and calcium-stimulated ATPase with resultant impaired excitation-contraction coupling and calcium overload which can also activate enzymes that causes further damage in the sarcolemma and sarcoplasmic reticulum [24]. Actually, these changes may persist after resolution of active ischemia [24].

In the current study, we evaluated the relationship between severity of CAD and QTD. We classified the patients into those with single, two, three vessels disease or left main stem disease or three vessels disease had QTD higher than those with two vessels disease who in turn have higher QTD than those with one vessel disease.

Notably, the relationship between severity of CAD and QTD has been demonstrated in post MI patients [25].

This study also assessed the effect of LV dysfunction or proximal LAD artery involvement on QTD in patients with CAD.

QTD did not increase significantly as severity of LV dysfunction increased LV dysfunction in patients with CAD may be due to myocardial stunning, hibernation [24] or scar due to previous MI.

In patients with previous MI, infarcted areas are associated with prolonged recovery times that, in the end, may lead to increased dispersion of repolarization compared with the base line state [24].

In TEAM-3 study [27] LV dysfunction was independently associated with increased QTD in patients with acute MI. However, Bodi et al [28] has revealed that QTD was not related to ejection fraction in patients within six months after an acute MI.

In fact, this contradiction mayy resulted from the differences in patients characteristics in diverse studies, as the study by Bodi and his Colleagues [28] has evaluated the effect of LV dysfunction on QTD in patients who have MI only, whereas the present study involves those with previous MI and those without history of MI.

The absence of significant increment of QTD in patients.

LAD artery supplies anterosuperior two thirds and the entire apical one third of ventricular septum, anterior LV free wall and the medial third of the anterior right ventricular free wall [33]. So critical stenosis in the proximal segment of this artery jeopardizes substantial area of myocardium.

Previously, Tikiz et al [21] showed that there is no relation between QTD and the pattern of CAD or the lesion localization in 119 patients with single vessel disease.

In our study, we used multiple regression analysis to identify variables independently affecting QTD. We included six variables: Age, hypertension, D.M., previous MI, severity of CAD and LV dysfunction.
Among these features, severity of CAD, LV dysfunction and previous MI are independently associated with increased QTD.

The impact of MI on QTD has been demonstrated also by Van de Loo et al [3] and Higham et al [29] who have revealed that QTD was higher in patients with acute MI than in those with unstable angina.

**Conclusions**

QTD is a simple ECG parameter, (which could be calculated easily at bed side) is significantly higher in patients with IHD in comparison to the normal persons and it increases with increased severity of CAD.

**Source of Funding-** Self

**Ethical Clearance –** Not required

**Conflict of Interest:** None

**References**


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Measurement of Interleukin 17a and Transforming Growth Factor-B1 and Its Relation to Disease Activity in Systemic Lupus Erythematosus Patients

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¹Researcher, College of science / University of Baghdad, ²Assist Prof, College of Science / University of Baghdad, ³Senior Specialist Doctor, Ibn Seena Hospital

Abstract

The study was done in January – may 2018, For measuring interleukin concentration 17-A and transforming growth factor-β1 in the serum of systemic lupus erythematosus (SLE) female patients(52 female) compared to the control group (36 healthy female).

An Eоzyme- Link ed Immun Osborne Assay (ELISA) was used to diagnose both IL-17A and TGF β1 in SLE patients and control. In case of IL-17A High significant in.crease (P < 0.0001) in SLE patients (38.54 pg / mL) compared with healthy control (20.24 pg / mL) was observed, while in patients with SLE, the transforming growth factor-β was (70.90 pg / mL) compared to healthy control (116.66 pg / mL) (P < 0.0001). To all SLE patients and control, The laboratory features include full blood count ,erythrocyte sedimentation rate ESR , ,an.d immunological tests like C3,C4,ANA and anti ds-DNA were done. ESR , WBC , ANA and Urea were significantly higher in SLE patient than con trol group (p.value 0.0001, 0.0001, 0.024, 0.0001 respectively) whereas Hemoglobin were significantly lower in SLE Patient than control group (p.value 0.0001) .C3&C4 were lower in SLE patients but with no significance value.

Key word s : Systemic Lupus erythematous ,Interleukin -17A, Trans forming growth factor-β1, disease activity.

Introduction

Systemic lupus erythematosus (SLE) is а chroz nic auto immune di sease that effect s many systems, organ s and tissues in the human bo dy such as kidney s, skin, cardiov.ascular system, lung.s, j.oints, muscles, and nervous system¹ . SLE has a broad variety of clinical presentations and various autoantibodies, most of which involve female of reproductive age.², ³. The duration of the disease differs with periods of remission and recurrence. ⁴. Although there has been important progress in understanding its etiopathogenesis over the previous few years, the actual cause of lupus is unknown. ⁴. Lupus pathogenesis includes many factors that are immunological, hormonal, hereditary and environmental. The incidence of disease differs among ethnic groups, among black females 1:250 and among white females 1:4300. ⁵,⁶.

In SLE pathogenesis, several main players such as cytokine over manufacturing ⁷,⁸, oxidative stress ⁹, and apoptosis s play a significant part. ⁴⁰

Cytokines plays a crucial role in the proeduction, maturaetion and diferentiation of immune cells. The effect of cytokine s on autoimmune SLE can not be continuously anticipated. ⁱ¹.

Interleukin 17 A is a multi-functional cyto.kine th.at influences the development of neutrophils and mediates T-helper-1 (Th1) and T-helper-2 (Th2) cytokines. ¹²,¹³,¹⁴.

In humans, IL-17A is mainly expressed through the T-helper-17 (Th17) subgroup of CD4 T-cells¹⁵, but it also produces neutrophils, CD8 + and double adverse (DN) T cells, and natural killer (NK) cells¹².

In vivo and in vitro production of IL-17 is mainly regulated by transform ing growth fact or beta-1(TGF-β) and interleukin-6 (IL-6) by the activ ation of the signal trans.ducer and the activator of transcription-3 (STAT-3) in mous e and hu.man models, respectively. ¹⁶,¹⁷.
Recent studies of IL-17 inhibition in achieve inanky,ising spo.ndylitis clinical advantage will raise concerns about the potential for inhibition of IL-17 in patients with SLE or in certain symptom types. (18,19) .

There are three tightly associated members in the cytokine family of transforming growth factor β (TGF-β): TGF-β1, TGF-β 2, and TGF-β 3. β 1 is of or m generated by lympho.cytes and mono.cytes with a significant immune regulatory function (20). TGF-β 1 is the most powerful naturally occurring immune suppressant, playing a main role in regulating cell proliferation and cell manufacturing through programmed cell death. TGF-β 1 is a important negative B-cell differentiation and proliferation regulator that inhibits the development of most immunoglobulin iso-types (21). It also co-stimulates the development of T-cells with down-regulating activities (22). TGF-β 1 can play a dual role in inflammatory immune-mediated disease development and progression. The production of TGF-β lymphocytes in SLE is decreased, which can predispose autoreactive T-cell activation and autoantibodies production. In autoimmune diseases, infiltration of target bodies such as T-cells or immune-complex autoantibody deposition triggers early inflammatory lesions that activate local production of anti-inflammatory cytokines such as TGF-β 1 by macrophages and kidney mesangial cells to combat inflammatory mesangial cells to prevent inflammation (23). Increased production of TGF-in 1 in tissues leads to local fibro.genesis and ultimately to severe organ damage (24). Any SLE treatment involving manipulation of TGF-β 1 should therefore be approached with caution, considering the effects on the entire cytokine network(25).

Patients and Method

Fifty-two female SLE patients were enrolled with 23-56 years of age (32.5 ± 1.1 years). They were referred during the period January – May 2018 for diagnosis and treatment to the Consultant Clinic at the Department of Rheumatology (Alyarmook Teaching Hospital and Baghdad Teaching Hospital). A control group of thirty six subjects were also included, and they were matched patients for gender and age where the age of patients and healthy individual range between 20 - 60 years.

Clinical Examination

Patients undergoing complete history and clinical examination including activity of disease , general locoumoter system, skin, cardiovgascular, thoracic, neurological and vascular examination.

Investigations

Investigations of each sample were done by full blood count, erythrocyte sedimentation rate, renal function tests, Com plment 3 , Com plment 4 ,AN A, and Anti double strand.ed DNA anti body.

Est Imation of Il-17A and Tgf B1

The patients ans controls serum were tested for IL-17A and TGF β levels. Sera was analyzed according to manufacturer’s protocols by sandwich enzyme-linked immuno.sorbent assay (ELISA). Quantikine® ELISA kit with. © Catalog Number D1700 used to check IL-17A with assay range: 11.373 - 59.326 pg/ml. TGF β assay by cusabio Co., LTD, the Catalogue Number:CSB-E04725h. Assay range: 22.508-151.321 pg/ml.

Statistical Analysis

Analysis of data was carried out using SPSS-25 (Statistical Social Science Packages- version 25). The significance of differing umeans (quantitative data) has been tested using Students-t-test to differentiate between two independent means or Paired-t-test for paired observation differences (or two dependent means), Or ANOVA test for distinction between more than two independent means. Measures the association closeness by correlation coefficient between twoou quantitative continuous variables. Kruskall Wallis one-way variance analysis (KW) test was used to compare the median for > 2 independent non-related samples. The P value was considered statistically significant less than 0.05.

Results

Table 1 shows the clinical features of patients with SLE compared to healthy control, there was no significant difference in (Creatinine ,Anti-dsDNA ,C4 ,and C3) of SLE patients and healthy control. SLE patients show highly result in ESR , WBC , ANA and Urea than control , whereas the test of Hemoglobin was lower in SLE patients than control.
Table (1): C3, C4, Anti-dsDNA, ANA, ESR, WBC, Hemoglobin, Platelets, Urea and Creatinine in the sera of patients with SLE and healthy control

<table>
<thead>
<tr>
<th></th>
<th>SLE patients</th>
<th>Healthy control</th>
<th>P.Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3 (IU/L)</td>
<td>0.93±0.42 (0.20-1.66)</td>
<td>1.08±0.33 (0.51-1.71)</td>
<td>0.094</td>
</tr>
<tr>
<td>C4 (IU/L)</td>
<td>0.37±0.17 (0.083-0.65)</td>
<td>0.39±0.15 (0.16-0.60)</td>
<td>0.746</td>
</tr>
<tr>
<td>Anti-dsDNA (IU/ml)</td>
<td>17.35±8.95 (6.48-36.90)</td>
<td>15.09±1.85 (12.1-17.80)</td>
<td>0.142</td>
</tr>
<tr>
<td>ANA (IU/ml)</td>
<td>3.25±1.92 (0.21-6.92)</td>
<td>1.09±0.06 (1.0-1.20)</td>
<td>0.0001*</td>
</tr>
<tr>
<td>ESR (mm/hour)</td>
<td>39.94±17.65 (15-88)</td>
<td>7.47±3.91 (2-17)</td>
<td>0.0001*</td>
</tr>
<tr>
<td>WBC (x103)</td>
<td>7.61±2.95 (3.8-14.30)</td>
<td>6.33±1.39 (4.0-9.10)</td>
<td>0.024*</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>11.20±1.94 (8.20-16.0)</td>
<td>14.16±1.58 (11.20-17.30)</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Platelets (x103)</td>
<td>283.28±137.50 (89-520)</td>
<td>269.94±63.52 (161-364)</td>
<td>0.603</td>
</tr>
<tr>
<td>Urea (mg/100 ml)</td>
<td>41.84±14.56 (19.0-74.0)</td>
<td>28.33±9.01 (16.0-44.0)</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Creatinine (umol/L)</td>
<td>1.03±0.34 (0.40-1.70)</td>
<td>1.00±0.27 (0.43-1.50)</td>
<td>0.641</td>
</tr>
</tbody>
</table>

Table (2) shows IL-17 A and TGF-β1 concentrations in patients with SLE and healthy control. Highly significant difference (P 0.01) was found in IL-17 A levels of patients & healthy control (38.54, 20.24 pg/mL) respectively, while TGF-β, the level in SLE patients 70.90 pg/mL and healthy control 116.66 pg/mL.

Table (2): Level of IL-17 A TGF-β in the sera of SLE patients & healthy control

<table>
<thead>
<tr>
<th></th>
<th>SLE patients</th>
<th>Healthy control</th>
<th>P.Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-17 A (pg/ ml)</td>
<td>38.54±5.41 (27.054-46.621)</td>
<td>20.24±5.28 (11.373-28.625)</td>
<td>0.0001*</td>
</tr>
<tr>
<td>TGF-β (pg/ml)</td>
<td>70.90±13.44 (50.644-94.343)</td>
<td>116.66±19.71 (90.514-151.32)</td>
<td>0.0001*</td>
</tr>
</tbody>
</table>
Discussion

Systemic lupus erythematosus is a complex autoimmune disease involving various aspects of the immune system. In addition to antibody production and immune complex deposition, emerging evidence suggests that cytokines can play a major role in the pathogenesis of SLE(26).

In our study, the comparison of TGF-β1 serum levels for both SLE patients and controls shows significant statistical difference whereas the patients with lower values than control p.value 0.001. This was consistent with the Becker-Merok et al'(27) outcomes, which revealed that SLE patients had reduced concentrations of TGF-β 1 (p 0.01) than controls.

Several cytokine abnormalities have been recorded in SLE and latest developments have revealed new understanding of autoimmune inflammatory reactions in cytokine regulation. The production of TGF-β1, the primary regulatory T cytokines that suppress inflammatory reaction, has been discovered to be profoundly deregulated in SLE patients in specific. They were therefore regarded as essential elements in the disease etiology (28). TGF-β 1 is a extremely pleiotropical cytokine that plays a significant role in immune homeostasis maintenance (29). TGF-β 1 includes many critical cellular procedures, including cell development, extracellular matrix formation, cell motility, hematopoiesis, apoptosis and immune function. (30). TGF-β 1 has strong anti-inflammatory and immunosuppressive properties, controlled by all immune cells activation, proliferation, differentiation and survival (31).

Also we found the IL-17A levels were significantly higher than controls. Other researchers showed elevated levels of IL-17A in the serum of SLE patients because these cytokines are generated by the immune-response T-helper lymphocyte, which can be deregulated in the impaired immune response. (32,33).

Increasing concentration of IL-17A in SLE patients will probably lead to the recruitment and activation of immune cells in target organs, thereby improving the immune response. In SLE patients, the immune environment is ideally suited for IL-17 A producing T-cell.

From this research we found that IL-17 A & TGF-β1 can also serve a protective function in SLE patients.

Conflict of Interest: There is no conflict of interest among the authors.

Funding: Self

Ethical Clearance: This study is ethically approved by the Institutional ethical Committee.

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26. Yap DY, Lai KN. Cytokines and their roles in the pathogenesis of systemic lupus erythematosus:


Device-associated Infections (DAIs) in ICU: Using new CDC/NHSN definitions

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Abstract

Surveillance of device-associated infections (DAIs) in intensive-care units (ICUs) is essential for a suitable healthcare strategy-planning. This study was conducted to determine the DAIs rate, microbial epidemiology, and antimicrobial resistance pattern (AMR) in ICU. Three major DAIs, ventilator associated pneumonia (VAP), catheter-associated urinary tract infection (CAUTI), and central line-associated bloodstream infection (CLABSI) were assessed prospectively during 6 months (2018 March 21 to September 22) in an 18-bed ICU of a tertiary teaching hospital in Tehran according to new CDC/NHSN definitions for surveillance of DAIs. The incidence of VAP, CAUTI, and CLABSI were 38.2, 8.9, and 13.5 per 1000 device-days respectively. The most organisms were Acinetobacter baumannii (34.9%), Klebsiella pneumonia (19%), E.coli (10.7%), Candida (10.7%), Enterococcus spp. (8.7%), Pseudomonas aeruginosa (8.7%), and Staphylococcus aureus (2.9%). Klebsiella pneumoniae carbapenemase (KPC)-producing was 47.4%. Acinetobacter resistance to Ampicillin-sulbactam and carbapenems was 80.6% and more than 90% respectively. Methicillin resistant Staphylococcus aureus (MRSA) was 66.6% and Vancomycin-resistant Enterococcus (VRE) was 88.9%. This study showed high incidence of DAIs and resistant organisms in the ICU. Preventive measures are essential to decrease the rates.

Key words: Device associated infection; antimicrobial resistance; intensive care unit

Introduction

Health care-associated infection (HAI) is an infection occurring in a patient during the process of care in a hospital or other health care facility which was not present or incubating at the time of admission (1,2). About 10% of patients admitted to hospitals are suffered from HAI (3).

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In intensive care units (ICUs), the commonly reported infections are ventilator-associated pneumonia (VAP), catheter-associated urinary tract infection (CAUTI), and central line-associated bloodstream infection (CLABSI) resulting in high mortality (4,5). In low and middle income countries, the frequency of hospital infections in ICU is 2-3 times higher than in high-income countries, and use of devices is much higher than in the United States (4,6).

Considering resistant microorganisms, hospital environments are the largest sources of vancomycin resistant enterococci (VRE), Methicillin-resistant Staphylococcus aureus (MRSA), and multidrug resistance (MDR) gram negative bacilli like Klebsiella.
pneumoniae; so the microbial epidemiology should be investigated and monitored (4,7).

In developing countries such as Iran, there are some limitations in accurate detection of infections, causative agents, and antimicrobial resistance (8). For the importance of HAIs and the antibiotic resistance patterns of microorganisms, this study was conducted to determine the DAIs rate, microbial epidemiology, and AMR in a general ICU in a tertiary collegiate hospital in Tehran, Iran.

**Material and Method**

In this study, 629 patients admitted to the general ICU from 21 March 2018 to 22 September 2018 were studied and analyzed for HAIs according to Center for Disease Control and Prevention/National Healthcare Safety Network (CDC/NHSN) case-definitions (2). Information of the patients affected with infections was recorded according to declaration of Helsinki on medical research ethics (9). In a pre-defined form designed to collect data, the patients’ demographic information, infection types, cultures results, and denominators (patient-days, ventilator days, central-line days, and urinary catheter days) were recorded. Finally, the rate of DAIs and microbial epidemiology were assessed based on the data coded on the questionnaire and entered into the SPSS software version 24. To display quantitative information, mean ± SD is used and prevalence is indicated for qualitative variables.

**Results**

The study was conducted in an 18-bed general ICU during a 6-month period in 2018. In this duration 692 patients were admitted which 385 (56%) were male and 307 (44%) were female.

The study showed that 103 patients (14% of all ICU admission) were infected with DAIs. Infection subtypes included VAP (53 cases, 51.5% of DAIs), CAUTI (27 cases, 26.2% of DAIs) and CLABSI (23 cases, 22.3% of DAIs). The patient-days were 3212 for this time period. Device-days were as follows: 1386 ventilator-days, 3018 urine-catheter-days, 1698 central-line-days. Device associated infection (DAI) rate or density was calculated for each subtype. This rate for VAP, CAUTI, and CLABSI per 1000 device-days was 38.7, 8.9, and 13.5 respectively (Table 1).

**Table 1: Device-associated infection (DAI) rate/density, and device utilization ratio (UR).**

<table>
<thead>
<tr>
<th></th>
<th>Frequency (No.)</th>
<th>Patient-days</th>
<th>Device-days</th>
<th>DAI Rate/Density</th>
<th>Device UR</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAP</td>
<td>53</td>
<td>3212</td>
<td>1386</td>
<td>38.7</td>
<td>0.43</td>
</tr>
<tr>
<td>CAUTI</td>
<td>27</td>
<td>3212</td>
<td>3018</td>
<td>8.9</td>
<td>0.94</td>
</tr>
<tr>
<td>CLABSI</td>
<td>23</td>
<td>3212</td>
<td>1698</td>
<td>13.5</td>
<td>0.53</td>
</tr>
</tbody>
</table>

VAP: ventilator-associated pneumonia; CAUTI: catheter-associated urinary tract infection; CLABSI: central line-associated bloodstream infection; UR: utilization ratio

The number of crude mortality among infected patients for VAP, CAUTI, and CLABSI was 27, 15 and 12 patients. According to these total 54 deaths (of 103 HAIs) the crude mortality rate was calculated to be 52%. Among causative agents, gram positive cocci in 14 cases (13.6%), gram negative bacilli in 78 cases (75.7%) and 11 candida isolates (10.7%) were reported (Table 2). And the patterns of antimicrobial resistance (AMR) demonstrate in Table 3.
Table 2: Prevalence of microorganisms in each DAI group.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>BSI No(%)</th>
<th>UTI No(%)</th>
<th>VAP No(%)</th>
<th>Total No(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram Positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>1 (4.3%)</td>
<td>0 (0%)</td>
<td>2 (3.8%)</td>
<td>3 (2.9%)</td>
</tr>
<tr>
<td>Streptococcus agalactiae</td>
<td>1 (4.3%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Enterococcus Spp.</td>
<td>8 (34.8%)</td>
<td>1 (3.7%)</td>
<td>0 (0%)</td>
<td>9 (8.7%)</td>
</tr>
<tr>
<td>Streptococcus pneumonia</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (1.9%)</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Gram Negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>1 (4.3%)</td>
<td>7 (25.9%)</td>
<td>3 (5.7)</td>
<td>11 (10.7)</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>3 (13.0%)</td>
<td>3 (11.1%)</td>
<td>13 (24.5)</td>
<td>19 (18)</td>
</tr>
<tr>
<td>Proteus Spp.</td>
<td>0 (0%)</td>
<td>1 (3.7%)</td>
<td>0 (0%)</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Enterobacter Spp.</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>2 (3.8)</td>
<td>2 (1.9)</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>0 (0%)</td>
<td>2 (7.4%)</td>
<td>7 (13.2)</td>
<td>9 (8.7)</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>7 (30.4%)</td>
<td>4 (14.8%)</td>
<td>25 (47.2)</td>
<td>36 (34.9)</td>
</tr>
<tr>
<td>Fungi</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida Spp.</td>
<td>2 (8.7%)</td>
<td>9 (33)</td>
<td>0 (0%)</td>
<td>11 (10)</td>
</tr>
<tr>
<td>Total</td>
<td>23 (100%)</td>
<td>27 (100%)</td>
<td>53 (100%)</td>
<td>103 (100%)</td>
</tr>
</tbody>
</table>
Table 3: The patterns of antimicrobial resistance (AMR) in microorganisms.

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of isolates</th>
<th>Antibiotic</th>
<th>Resistance No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>3</td>
<td>Vancomycin</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cefoxitin</td>
<td>2 (66.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clindamycin</td>
<td>2 (66.6)</td>
</tr>
<tr>
<td>Enterococcus spp</td>
<td>9</td>
<td>Ampicillin</td>
<td>4 (44.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vancomycin</td>
<td>8 (88.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Linezolid</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>19</td>
<td>Imipenem</td>
<td>9 (47.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Piperacillin-Tazobactam</td>
<td>14 (73.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ceftazidime</td>
<td>15 (78.9)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>11</td>
<td>Imipenem</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ceftriaxone</td>
<td>5 (45.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ciprofloxacin</td>
<td>5 (45.4)</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>36</td>
<td>Ampicillin-Sulbactam</td>
<td>29 (80.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Imipenem</td>
<td>34 (94.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Colistin</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>9</td>
<td>Amikacin</td>
<td>4 (44.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gentamicin</td>
<td>7 (77.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Piperacillin-Tazobactam</td>
<td>4 (44.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ceftazidime</td>
<td>4 (44.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ciprofloxacin</td>
<td>4 (44.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Imipenem</td>
<td>7 (77.8)</td>
</tr>
</tbody>
</table>
Discussion

In this study, we investigated the device-associated infections and the microbial pattern in a general ICU in a tertiary collegiate hospital in Tehran, Iran. The incidence for VAP, CAUTI, and CLABSI per 1000 device-days was 38.7, 8.9 and 13.5, respectively. According to the World Health Organization (WHO) report, the incidence for VAP, CAUTI, and CLABSI per 1000 device-days in developing countries is 23.9, 8.8, and 12.2(1). Comparing with this report, the rate of VAP is clearly higher in our ICU. The reason might be failure to fully design and implement an appropriate bundle for infection control of VAP in the ICU. In the International Nosocomial Infection Control Consortium (INICC) report for DAIs in ICUs of 50 low- and middle-income countries, the incidence density for VAP, CAUTI and CLABSI per 1000 device-days was 13.1, 5.07 and 4.1 respectively(10). In our study all DAIs rate were higher; might be for weakly implement of infection control components such as low hand hygiene compliance, inappropriate contact isolation, and etc. Afhami et al in a recent multi-center study in Tehran showed the rate of 21, 7.4, and 10.2 for VAP, CAUTI, and CLABSI in 1000 device-days(4). According to that multi-center study, it seems even in compare with similar ICUs in our local region, the rate of VAP in our ICU is higher and we should consider more preventive and control measures.

For causative agents and antimicrobial resistance, this study showed that overall in DAIs gram negative bacilli like Acinetobacter baumannii and Klebsiella pneumoniae were the most common organisms, and they were often multidrug resistance. In European Centers for Disease Control and Prevention (ECDC) report for ICUs, the most frequently isolated microorganism was Pseudomonas aeruginosa in pneumonia, coagulase-negative staphylococci in bloodstream infecions, and Escherichia coli in urinary tract infections(11). There was also in this report that 30% of Staphylococcus aureus isolates were oxacillin-resistant (MRSA). Resistance to third-generation cephalosporins was reported in 18% of E. coli isolates, 38% of Klebsiella spp. isolates and 32% of Enterobacter spp. isolates. Carbapenem resistance was reported in 11% of Klebsiella spp. isolates, 27% of P. aeruginosa isolates and 66% of Acinetobacter baumannii isolates (11). Comparing with ECDC report, in our study drug-resistance was much higher, representing the necessity of paying more attention to AMR and planning to prevent creation of such resistant microorganisms and control the spread of them in our ICU. In INICC report, the same microorganisms reported but the resistance level was lower than in our study (10).

There were some limitations to this study. The ICU was in a tertiary collegiate hospital; therefore, the patients with severe underlying diseases managed and required more intensive care, so the infection rate in our study may have been higher than the overall rate in the region.

Conclusion

This study showed high incidence of DAIs and resistant organisms in the ICU. Preventive measures are essential to decrease the rates.

Ethical Clearance: This article is the result of a research project (General Practitioner) approved by the Ethics Committee in Tehran University of Medical Science, Tehran, Iran.

Conflict of Interest: The author(s) declared no potential conflicts of interest.

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References


NOAEL Benzene in White Mice as the Basis for Determining the Safe Limit of Benzene Concentration in the Pulogadung Shoe Industry Home in Jakarta

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Abstract

The shoe industry in its operational activities is very dependent on the quality of the material, one of which is glue containing benzene, the industrial workers have a risk of exposure to benzene. The purpose of this study was to determine the safe concentration of benzene in the leather shoe industry in the small industrial village of Pulogadung, Jakarta. This research is descriptive research. The population in this study were shoe workers who worked on the installation of sole 1 and sole 2, which amounted to 16 people. The results of the study were quantitatively analyzed to determine the safe concentration of benzene for workers obtained from data on benzene concentrations in the workplace, worker height, worker weight, rats’ weight, worker respiration rate, length of work, worker body surface area, surface area mice body, highest dose of toxin without effect on experimental animals (NOAEL), Km factor in animals (Animal Km), factor Km in workers (Human Km), and safe limit for toxin dose for workers (RfC). The measurement results of the concentration of benzene in the sole 1 section were 0.19 ppm and in the sole 2 part was 0.06 ppm, which means that the concentration of benzene is still below the Threshold Value according to Minister of Manpower Regulation Number 13 of 2011 at 0.5 ppm. Based on the calculations that have been done, the safe limit value is 0.021 ppm. Based on the minimum risk level (MRL), the concentration of benzene every day that can cause acute effects is 0.009 ppm and that can cause chronic effects is 0.003 ppm. From these standards, it can be seen that the concentration of benzene in the leather shoe industry has the potential to have an impact on the health of workers. Control measures are needed to see potential health problems that workers can experience because of the benzene. Recommendations to the leather shoe industry are to periodically monitor benzene levels in the air, conduct health checks on workers, and use personal protective equipment on workers such as masks to reduce levels of inhaled benzene into workers’ bodies.

Keywords: Benzene, NOAEL, shoe industry

Introduction

The informal industry sector is an unorganized, irregular, and some legal but not registered sector1. One type of informal business is the shoe industry. The materials used in making shoes are leather, footwear, and glue to glue. The increasing number of shoe industries, the more glue is used to produce these shoes. Hazardous substances in glues are volatile organic compounds (benzene, toluene, and xylene) which fall into the category of volatile VOC (Volatile Organic Compound)2. Based on the research conducted by the Director General of Indonesian Public Health, stated that the organic solevents in the glue were 70% toluene and more than 1-2% benzene solevents3.

Benzene is a chemical that is dangerous and carcinogenic to humans and is a colorless and volatile liquid. Benzene is widely used in the rubber industry, oil refining, shoe factories, chemical plants and other
The glue used in the shoe industry contains benzene which affects benzene exposure in the shoe industry. The permissible levels of benzene exposure according to several world organizations are 1 ppm determined by the National Institute for Occupational Safety and Health5 and Occupational Health and Safety Assessment Series6 and 0.5 ppm set by the American Conference of Governmental Industrial Hygienists7.

Indonesia itself has set a limit exposure in the benzene work environment that is set in the Threshold Limit Value (NAB) of benzene of 0.5 ppm in accordance with the Minister of Manpower and Transmigration Regulation of the Republic of Indonesia number PER/1/ MEN/ X/2011 concerning Factor Threshold Values Physics and Chemistry at Work8.

Several health cases related to benzene exposure have been found in several countries. Some cases due to the use of benzene in the shoe industry were in 1946-1956 in the United States there were 107 cases due to the concentration of benzene exposure which exceeded 400 ppm. Of these cases, hemopathy and thrombocytopenia were found9. In 1945-1955 there were 125 cases of platelet decline and abnormal liver function due to exposure to benzene which exceeded 400 ppm in the shoe industry. Whereas in 1948, the API (American Petroleum Institute) published that benzene is certain to cause leukemia and there is no small tolerance (zero ppm level) to benzene emissions10.

Determination of safe limits of benzene concentration in the work environment must involve knowledge of worker respiration rate (R), reference concentration (RfC) benzene, duration of work / day (tE), frequency of work each year (fE), duration of work (years) weight (Wb) and the average cancer year (70 years x 365 days) and non-cancer (30 years x 365 days)11.

This study aims to determine the magnitude of the environmental health risks of benzene exposure to workers in the shoe industry in Pulogadung Small Industry Village using an environmental health risk analysis approach. The population in this study were shoe workers who worked on the installation of solee 1 and solee 2, which amounted to 16 people.

The design of the research is to collect initial data related to work processes (chemicals in the area) and the number of workers. Furthermore, collecting primary data is the concentration of benzene in the workplace air, length of work time, and worker weight. In addition, secondary data collection was also carried out on experimental animals, namely the weight of white mice. The type of data in this study is primary data, obtained through questionnaires and observations as well as measurements of benzene in the air of the respondent’s workplace.

The variables in this study were benzene concentration in the workplace, worker height, worker body weight, rats’ weight, worker respiration rate, length of work, body surface area, surface area of the rat body, highest dose of toxin without effect on experimental animals (NOAEL), Km factor in animals (Animal Km), Km factor in workers (Human Km), safe limit of toxin dose for workers (RfC), and benzene concentration in safe air for workers (Safe C).

Data analysis in this study was carried out by using quantitative data analysis manually to determine the safe concentration of benzene for workers in the pulogadung leather shoes factory working environment.

**FINDINGS**

1. Characteristics of Experimental Animals and Surface Area of Animals Experiments (White Mice)

Toxicity can be interpreted as the ability of poisons (molecules) to cause damage if it enters the body and the location of organs susceptible to it. In this study, the implementation of a toxicity test using experimental animals namely white rats. This is done considering the human response to toxicity qualitatively is the same as the response of animals, so this fact is the basis of extrapolation from animal to human data.
Based on these data, it can be calculated the body surface area of white mice with

Animal BSA = 0.09 \cdot W^{0.67}

Description:

BSA : Body Surface Area (m²)
W : Weight (kg)

2. Characteristics of Workers, Worker’s Body Surface Area, and Worker’s Respiratory Rate

In this study, worker characteristics include weight and duration working time. The sample in this study were 16 respondents, namely workers in the pulogadung leather shoe factory.

Based on data on worker weight and height of workers, the body surface area and the rate of respiration of workers can be calculated using the following formula.

\[
\text{BSA}_{\text{human}} = \sqrt{\frac{W \cdot h}{3600}}
\]

Specification:
BSA: Body surface area (m²)
W : Weight (kg)
h : Height (cm)

B. Respiratory rate of worker

\[
\text{BR} = \frac{5.3 \cdot (\ln W) - 6.9}{24}
\]

Description:
BR : Breathing rate (m³/h)
W : Weight (kg)

From the calculation of the body surface area and the respiratory rate of workers, it is known that the average body surface area of workers is 1.53 m² and the average respiration rate of workers is 0.59 m³/hour.

3. Benzene Concentration

The measurement results of benzene concentration at two points in the leather shoes industry showed that the measurement results in the sole 1 section were 0.63 mg / m³ (0.19 ppm) and in the sole 2 section 0.29 mg / m³ (0.06 ppm)
Measurement results done, it is known that the concentration of benzene in the working environment of pulogadung leather shoes in sole 1 part is 0.19 ppm and in sole 2 parts 0.06 ppm. Based on the Minister of Manpower and Transmigration Regulation No. 13 of 2011 concerning the threshold value of physical factors and chemical factors in the workplace for concentrations of Benzene of 0.5 ppm so that the concentration of benzene in the shoe industry, the concentration of Benzene is below the NAB. However, the concentration of benzene is above the Minimum Risk Level (MRL), the level of benzene inhalation exposure set by ATSDR, i.e., for acute exposure (≤14 days) = 0.009 ppm, moderate exposure (15-364 days) = 0.006 ppm, and exposure chronic (≥365 days) = 0.003 ppm.

4. Animal Km and Human Km

A. Animal Km

\[ \text{Animal Km} = \frac{W_{\text{animal}}}{\text{BSA}_{\text{animal}}} \]

Description:
- Animal Km: Km factor in animals
- W: Experimental animal weight (white mouse)
- BSA: Body Surface Area of experimental animals (White mice)

Table 4. Results of Calculation of Animal Km in Experimental Animals (White Mice)

<table>
<thead>
<tr>
<th>Experimental Animals (White Mice)</th>
<th>Animal Km</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.81420952</td>
</tr>
<tr>
<td>2</td>
<td>5.81420952</td>
</tr>
<tr>
<td>3</td>
<td>5.82102947</td>
</tr>
<tr>
<td>4</td>
<td>5.82102947</td>
</tr>
<tr>
<td>5</td>
<td>5.80052067</td>
</tr>
<tr>
<td>6</td>
<td>5.81420952</td>
</tr>
<tr>
<td>average</td>
<td>5.81</td>
</tr>
</tbody>
</table>

The calculation results of Animal Km in animal experiments white rats were 5.81.

B. Human Human Km

\[ Km_{\text{human}} = \frac{W_{\text{human}}}{\text{BSA}_{\text{human}}} \]

Description:
- Human Km: Km factor in human/worker
- W: worker weight
- BSA: Body Surface Area worker

Table 5. Human Km Calculation Results for Leather Shoe Industry Workers

<table>
<thead>
<tr>
<th>Workers</th>
<th>HumanKm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30.07</td>
</tr>
<tr>
<td>2</td>
<td>37.8</td>
</tr>
<tr>
<td>3</td>
<td>35.5</td>
</tr>
<tr>
<td>4</td>
<td>32.6</td>
</tr>
<tr>
<td>5</td>
<td>37.6</td>
</tr>
<tr>
<td>6</td>
<td>35.5</td>
</tr>
<tr>
<td>7</td>
<td>32.4</td>
</tr>
<tr>
<td>8</td>
<td>35.06</td>
</tr>
<tr>
<td>9</td>
<td>32.4</td>
</tr>
<tr>
<td>10</td>
<td>38.8</td>
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<tr>
<td>11</td>
<td>37.2</td>
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<td>12</td>
<td>30.07</td>
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<td>13</td>
<td>37.2</td>
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<td>14</td>
<td>35.7</td>
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<tr>
<td>15</td>
<td>35.5</td>
</tr>
<tr>
<td>16</td>
<td>34.9</td>
</tr>
<tr>
<td>average</td>
<td>34.9</td>
</tr>
</tbody>
</table>

The calculation results of Human Km are shown in table 5. Average Human Km for Leather Shoe Industry workers which is 34.9.

5. NOAEL

NOAEL is the highest dose of a substance in statistical or biological chronic or subchronic toxicity studies that do not show detrimental effects on test animals. Toxicology testing can be used to calculate No Observed Adverse Effect Level (NOAEL) and is
useful for clinical trials. To determine the safe limit of concentration of a chemical begins with the toxicity test No Observed Adverse Effect Level (NOAEL).

Swaen (2010) states that benzene NOAEL is 3.0 mg / m³ or equivalent to 0.022 mg / kg.

\[
NOAEL_{benzene} = \frac{3 \times 0.0013 \times 8}{0.1405} = 0.022 \text{ mg / kg}
\]

6. Safe Human Dose

\[
RF_C = \frac{NOAEL_{Animal \text{ Km}}}{NOAEL_{Human \text{ Km}}}
\]

**Description:**
- **RF_C**: Safe human dose (mg / kg)
- **Animal Km**: Km factor in animals
- **Human Km**: Km factor in humans

Based on these equations, the calculation of RF_C obtained is

\[
RF_C = \frac{5.81}{34.9} = 0.003 \text{ mg / kg}
\]

7. Limit of Safe Level of Benzene Concentration

Determination of safe limits of benzene concentration in the work environment of the leather shoes industry uses the following formula (William, 1985; Soemirat, 2003; Davis, 1991).

\[
C_{safe} = \frac{(SHD)(W)}{(O)(BR)(t)} \text{ mg / m}^3
\]

To convert units of mg / m³ to ppm the following formula is used.

\[
C_{safe} = \frac{11 \text{ mg / m}^3}{(MW)} \times 24.5 \text{ ppm}
\]

**Description:**
- **C_safe**: concentration of toxin in the air is safe for workers (mg / m³)
- **SHD**: Safe Human Dose (mg / kg)
- **W**: Weight (kg)
- **O**: % of substances absorbed by the lung
- **BR**: Human respiratory rate (m³ / hour)
- **t**: Duration of working time (hours)
- **MW**: Molecular Weight / Molecular Weight

Based on the above equation, the calculation results of the concentration of safe concentration of benzene in the Leather Shoes Industry is:

\[
C_{safe} = \frac{(0.003)(53.75)}{(50%)(0.59)(6)} = 0.006 \text{ mg / m}^3
\]

\[
C_{safe} = \frac{0.068 \times 24.45}{75.11} = 0.021 \text{ ppm}
\]

The results of the calculation of the concentration of safe levels of benzene in the air for workers above can be used to predict the concentration of toxins in the air in a safe work environment for workers and to be compared with NAB that has been set by various good institutions by Kemenakertrans, National Standardization Agency, ACGIH, NIOSH, and OSHA.

Benzene in the shoe industry must be identified to determine the level of exposure that can cause effects on shoe workers. What can be done to reduce benzene exposure to workers is to use PPE regularly such as masks, especially during the bonding process. Another approach is taken by eating foods rich in detoxification enzymes, especially GSH which is usually found in most vegetables such as asparagus, spinach, broccoli, garlic, kale, and onions12.

**Conclusion**

The results of the measurement of benzene concentration in the Leather Shoes Industry in Pulogadung Small Industrial Village, Jakarta in the sole 1 section are 0.19 ppm and in the sole 2 part is 0.06 ppm. Based on the Minister of Manpower and Transmigration Regulation Number 13 of 2011, the threshold value of benzene is 0.5 ppm. From the results of measurements in this study when compared with the threshold value then it is still below the set threshold value.

Based on the minimum risk level (MRL) of ATSDR 2007, the concentration of benzene every day that can cause acute effects is 0.009 ppm and that can cause chronic effects is 0.003 ppm. From these standards, it can be seen that the concentration of benzene in the leather shoe industry has the potential to have an impact on the health of workers.

Control measures are needed to see potential health problems that workers can experience because of the benzene. Recommendations to the leather shoe industry are to periodically monitor benzene levels in the air, conduct health checks on workers, and use personal protective equipment on workers such as masks to reduce levels of inhaled benzene into workers’ bodies.

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**Source of Funding:** The source of this research costs from self.
**Ethical Clearance:** The study was approved by the Institutional Ethical Board of Faculty of Public Health, Airlangga University.

All subjects were fully informed about the procedures and objectives of this study each subject prior to the study signed an informed consent form.

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The Relationship between Heat Stress and Dehydration in the Continuous Casting Machine Section of Pt X

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Abstract

One of the hazards in the CCM (Continuous Casting Machine) section of PT X is the heat stress. The heat stress is predominantly caused by hot metal liquids processed before being compacted into billets. Heat Stress causes workers sweating a lot and leads to dehydration. The purpose of this study is to find the correlation between heat stress and dehydration in CCM (Continuous Casting Machine) section of PT X. It is an observational study and a cross-sectional study due to time examined. The tools to measure heat stress is Heat Stress Apparatus Quest Temp 36 and measurement of work dehydration is by observing the color of the respondent’s urine compared to the urine color table. This study has a sample of 30 workers exposed to the hot work climate in CCM (Continuous Casting Machine) section of PT X. The data analysis techniques used are univariate and bivariate analysis techniques. The results show that the highest working climate in the place is 42.3 °C and of the 30 respondents studied, there are 28 respondents who are dehydrated, ranging from mild to severe. By using the chi-square test statistical calculations to see the correlation, p is 0.045 where p < α means there is correlation between the work climate and work dehydration. Suggestions that can be given to companies to overcome heat stress problems are by adding fan blowers in the workplace and providing electrolyte salts added to the workers’ drinks.

Keywords: Heat Stress, Relationship, Dehydration, Continuous Casting Machine, PT X

Introduction

Every company has hazards in their workplaces. One of the factors of hazard in the workplace of a company is the work climate. If work climate exceeds the allowable Threshold Limit Value (TLV) that can affect workers, workers can experience a work-related illness caused by the work climate. If the worker suffers from a disease due to the work climate, it will have an impact of decreasing productivity in the company.

Heat Stress is a combination and attachment of work temperature, air humidity, wind speed, and radiation temperature in the workplace. Work climate that is uncomfortable and discordance with the standards of workers and provisions will lessen workers’ capacity resulting a decline in efficiency and productivity of work(1). According to the Minister of Manpower and Transmigration Regulation, the work climate is the result of a combination of temperature, humidity, movement speed of air and radiant heat due to the level of heat dissipation from the workforce as a result of work(2).

The temperature in the workplace can be influenced by machinery and work environment factors. As long as the worker’s body is active, the body will naturally adjust the temperature received through the process of heat loss. The comfortable temperature for Indonesians at work is between 24-26 °C. When workers are exposed to hot temperature, it will reduce agility, prolong reaction time and slow down decision-making time, disrupt the accuracy of the brain’s work, interfere motor sensory nerve coordination, and provoke emotion. The high-risk working environment, especially the hot work climate, can endanger the safety and health of the workers, so it is necessary to make work time adjustments and safeguards to overcome the hazards at work that might happen(3). When the heat in the workplace exceeds the limit and disturbs the comfort of workers, it can cause

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Heat stress is a limit of the body’s ability as a result of receiving heat exposure from the combined contributions while doing a job and the factors that exist in the environment (such as air temperature, humidity, air movement, and heat transfer radiation) and clothing used when working. Mild or moderate heat stress conditions can cause uncomfortable condition for workers, and disturb the performance and safety, although this does not cause harm to workers’ health\textsuperscript{(4)}.

Dehydration is a health disorder caused by lack of fluids, especially Na salt. The effect of exposure to hot temperature and accompanied by excessive sweating will result in the loss of sodium salt. If someone loses fluid $<1.5\%$, the symptoms still do not appear, but fatigue will appear earlier with dry mouth. In such condition, workers only need to be given rest time in a cooler place and given sweat relieving powder\textsuperscript{(5)}.

In a study conducted by Nindi Puspita in 2014, the result of the effect calculation of hot work climate on dehydration by SPSS Mann Whitney test showed that the $p$ value was 0.023 or $p \leq 0.05$. This value indicates that there is a significant influence between the work climate and dehydration\textsuperscript{(6)}.

PT. X is a production site whose scope of business is in the field of hot roll steel plate. In the production process, it has exposure to hot temperatures affecting the company’s workers. The example of impacts that can be caused by hot temperature exposure to workers is dehydration. Therefore, researcher will analyze the correlation between heat stress and dehydration.

**Material and Method**

Based on the type of research and the method of data collection, this research is an observational study with cross sectional study. The sample in this study are 30 workers who work in the CCM (Continuous Casting Machine) section of PT X and exposed to hot work climate in their workplace. This research was conducted in one of the Surabaya regional companies, East Java, which is engaged in the production of hot rolled steel, in this case called PT X. Data collection was carried out in February 2019 for one week.

Secondary data is taken from company data that contains a general description of the company and a list of PT X workers. The first primary data collection is through interviews to find out about the identity and characteristics of the respondents (ages, work periods, and nutritional status). The heat stress measurements were carried out in the workplace that has heat exposure at PT X, the tool used is Questemp 36 heatstress monitor by looking at the Wet Bulb Globe Temperature Index (WBGT Index), after the WBGT results are obtained, they are compared to workload and working time to determine whether the results are more or less than the Threshold Value Limit (TLV) determined by law.

Measuring the level of dehydration is done on workers in areas that have heat exposure in PT. X, the measurement done is observing the color of the respondent’s urine after work. The categories of dehydration work are not dehydrated, mild dehydration, moderate dehydration, and severe dehydration. The variables studied are the characteristics of workers consisted of age, years of service, nutritional status and heat stress with work dehydration. Data is processed by using statistical chi-square test for data with nominal data scale and spearman correlation test for ordinal data scale.

**Finding**

**Ages**

The age group of workers working in the CCM (Continuous Casting Machine) section if PT X is divided into 3; $\leq$35 years, 35–50 years, and $>$50 years. The results of the age group $\leq$35 years are 16 workers, groups of working period 11-20 years are 5 workers, working period 21-30 years are 4 workers and groups working period $>$30 years are 5 workers.

**Work Periods**

The working period of the workers in CCM (Continuous Casting Machine) section of PT X is divided into 4; $\leq$10 years, 11-20 years, 21-30 years, and $>$30 years. The results of working period $\leq$10 years are 16 workers, groups of working period 11-20 years are 5 workers, working period 21-30 years are 4 workers and groups working period $>$30 years are 5 workers.

**Nutritional Status**

The nutritional status group of workers in the CCM (Continuous Casting Machine) section of PT X is divided into 5, namely mild lack of weight, severe lack of weight, normal weight, mild overweight, and heavy overweight. The results of the normal nutritional status
group are 15 workers, the mild overweight status group are 11 workers, and the severe overweight group are 4 workers.

Heat Stress

In measuring heat stress, determining the measurement point is based on the work location of the respondent. There are 3 points, namely the point A CCM located in front of the CCM Control room, point B CCM located near CCM Ladle, and point C CCM is near the Horizontal CCM Burner. The lowest Wet Bulb Globe Temperature Index (WBGT) results is 30.1 °C at Point A CCM which located in front of the CCM control room and the highest Wet Bulb Globe Temperature Index (WBGT) is 42.3 °C at point B CCM near the Horizontal CCM Burner. Setting working hours on the CCM section of PT. X is 25-50%, so the TLV for heat stress is 32 °C for light workloads, 30 °C for medium workloads, and 29 °C for heavy workloads.

Work Dehydration

Measurements of work dehydration is achieved by observation of after working color urine. Furthermore, the measurement results are classified into 4, namely not dehydrated, mild dehydration, moderate dehydration and severe dehydration. The results of not dehydrated group is 2 workers, mild dehydration group is 8 workers, moderately dehydration is 16 workers and severe dehydration is 4 workers.

Relationship Between Age Of Workers And Work Dehydration

Table 1. Cross Tabulation of Relationship between Age and Work Dehydration

<table>
<thead>
<tr>
<th>Ages</th>
<th>Work Dehydration Status</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not Dehydrated</td>
<td>Mild</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>≤ 35 years old</td>
<td>2</td>
<td>6,7%</td>
</tr>
<tr>
<td>36 – 50 years old</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>&gt; 50 years old</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>6,7%</td>
</tr>
</tbody>
</table>

The table above shows that most workers aged ≤35 years old experience moderate work dehydration as many as 8 workers with a percentage of 26.7%. Most workers 36-50 years old also experience moderate work dehydration by 5 workers with a percentage of 16.7%. Then the most work dehydration status experienced by workers aged >50 years is moderate work and severe dehydration are 3 workers with a percentage of 10%. The results of statistical tests, namely the Spearman correlation test showed that there is a significant connection between ages and work dehydration with a value of p = 0.000 <α = 0.05 and the correlation coefficient value showed a number of 0.619, which means the age of workers has strong level of correlation strength with dehydration.
Relationship Between Work Period Of Workers And Work Dehydration

Table 2. Cross Tabulation of Relationship between Work Period and Dehydration Work

<table>
<thead>
<tr>
<th>Work Period</th>
<th>Not Dehydrated</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>≤ 10 years</td>
<td>2</td>
<td>6,7%</td>
<td>5</td>
<td>16,7%</td>
<td>8</td>
</tr>
<tr>
<td>11–20 years</td>
<td>0</td>
<td>0%</td>
<td>1</td>
<td>3,3%</td>
<td>4</td>
</tr>
<tr>
<td>21-30 years</td>
<td>0</td>
<td>0%</td>
<td>2</td>
<td>6,7%</td>
<td>2</td>
</tr>
<tr>
<td>&gt; 30 years</td>
<td>0</td>
<td>0%</td>
<td>0</td>
<td>0%</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>6,7%</td>
<td>8</td>
<td>26,7%</td>
<td>16</td>
</tr>
</tbody>
</table>

The table above shows that most work dehydration status experienced by workers with a work period ≤ 10 years are moderate work dehydration with 8 workers by the percentage of 26.7%. The most work status experienced by workers with a working period of 11-20 years experienced moderate work dehydration is 4 workers with the percentage of 13.3%. The most work dehydration status of work experienced by 21-30 years working period is moderate dehydration and heavy with 2 workers by 6.7%. The most work dehydration status experienced by workers with working period >30 years is severe work dehydration by 3 workers with the percentage of 10%.

The results of statistical tests, namely Spearman correlation test shows that there is a significant correlation between work period and work dehydration. with a value of $p = 0.047 < \alpha = 0.05$ and the correlation coefficient showed a number of 0.365 which meanswork period has a low level of correlation strength with dehydration.

Relationship Between Nutritional Status Of Workers And Work Dehydration

Table 3. Cross Tabulation of Relationship between Nutritional Status and Work Dehydration

<table>
<thead>
<tr>
<th>Nutritional Status</th>
<th>Not Dehydrated</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Normal</td>
<td>2</td>
<td>6,7%</td>
<td>4</td>
<td>13,3%</td>
<td>9</td>
</tr>
<tr>
<td>Mild Level Overweight</td>
<td>0</td>
<td>0%</td>
<td>4</td>
<td>13,3%</td>
<td>5</td>
</tr>
<tr>
<td>Severe Level Overweight</td>
<td>0</td>
<td>0%</td>
<td>0</td>
<td>0%</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>6,7%</td>
<td>8</td>
<td>26,7%</td>
<td>14</td>
</tr>
</tbody>
</table>
The table above shows that the most work dehydration status experienced by workers with normal nutritional status was moderate work dehydration status as many as 9 workers with percentage is 30%. The most work dehydration status experienced by workers with mild level overweight nutrition status were moderate work dehydration as many as 5 workers with a percentage of 26.7%. The most work dehydration status experienced by workers with severe level overweight nutritional status were moderate working and severe dehydration as many as 2 workers with a percentage of 6.7%.

The results of statistical tests, namely the Spearman correlation test showed that there is a significant relationship between nutritional status and work dehydration with a value of $p = 0.034 < \alpha = 0.05$ and the correlation coefficient showed a number of 0.388 which means the age of workers has a weak level relationship with dehydration.

Relationship Between Heat Stress And Work Dehydration

Table 4. Cross Tabulation of Relationship between Nutritional Status and Dehydration Work

<table>
<thead>
<tr>
<th>Heat Stress</th>
<th>Work Dehydration Status</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not dehydrated</td>
<td>Mild</td>
</tr>
<tr>
<td>≤ TLV</td>
<td>2</td>
<td>6.7%</td>
</tr>
<tr>
<td>&gt; TLV</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>6.7%</td>
</tr>
</tbody>
</table>
The table above shows that heat stress measurements ≤TLV (Threshold Limit Values), the most workers experience mild and moderate dehydration are 4 workers with the percentage of 13.3%. The heat stress measurement >TLV, the most workers experience moderate work dehydration are 12 workers with a percentage of 40%. By using the chi-square test statistical calculations to see the relationship produced p is 0.045 where p <α, it means that heat stress and work dehydration are correlated. This research is supported by research conducted by Sari (2014) there is a relationship between hot work climate and dehydration in the boiler part of the workforce at PT. Albasia Sejahtera Mandiri Semarang Regency with a p value of 0.023 <0.05(7).

This research is in line with the theory from Suma’mur (2009), the workforce working in a workplace that exceeds the TLV of the work climate can experience the effects of heat stress. The effect of heat stress occurs as a result of the body’s process of maintaining body heat unsuccessfully. The effects of heat stress can be in the form of subjective complaints due to heat stress such as complaining of heat, sweating, always thirsty, feeling bad and loss of appetite caused by loss of fluid from the body by evaporation of sweat(5).

Exposure to a physical work environment such as heat stress can lead to health problems, one of which is dehydration. Consumption of drinking water needs to be considered because lack of fluids can cause dehydration as well. Prolonged dehydration can cause impaired kidney function. In addition, dehydration can also affect one’s weight due to sweat and urine that comes out during activities. Dehydration is excessive loss of body fluids due to insufficient fluid replacement intake that does not meet the body’s needs and an increase in water expenditure(8).

**Conclusion**

The results show that the heat stress measurement in CCM (Continuous Casting Machine) section of PT X already pass the NAB with a moderate workload and 25-50% working hours setting, namely the WBGT (Wet Bulb Globe Temperature Index) exceeding 30°C. The measurement of worker work dehydration in CCM (Continuous Casting Machine) section of PT X has the most moderate dehydration with 16 workers. There is a relationship between heat stress and dehydration work in the CCM (Continuous Casting Machine) section of PT X.

Advice that can be given to the company is that the company provides several places for workers to take their drinks close to their work area, when workers feel thirst or dehydrated, they can easily take the drinking water. Adding electrolyte salt to the workers’ drinks also strongly recommended.

**Funding**: Self – funding

**Conflict of Interest**: There are not any of conflicts amongst the authors

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The Effect of CHOP Chemotherapy Implementation Towards Reduced Global Longitudinal Strain in Non-Hodgkin Lymphoma

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Abstract

Background: Doxorubicin in CHOP regimen is still the golden standard of first-line chemotherapy in patients with NHL (Non-Hodgkin Lymphoma); however, it has enormous cardiotoxicity side-effects.

Objective: This study aims to investigate the effect of CHOP chemotherapy implementation towards the GLS reduction in cumulative dosage of doxorubicin ≥ 100 mg/m.

Method: This study was pre experimental analysis with modified cohort. The subjects were observed for 1 month, since the first chemotherapy until 1 week after the second chemotherapy. The echocardiogram procedure with ejection fraction and global longitudinal strain was conducted twice which was before the first chemotherapy and 1 week after the second chemotherapy.

Results: The implementation of CHOP therapy with the cumulative dosage of ≥100 mg/m² has resulted in reduced GLS from 19.62% to -18.03%. The reduction of GLS>10% was 40% while the LVEF has not been obtained any reduction > 10%. The case of subclinical left ventricular dysfunctions was attained 16%.

Conclusion: The implementation of CHOP chemotherapy with the dosage of ≥100mg/m² has been proved to cause reduced GLS and subclinical left ventricular dysfunctions.

Keywords: Global longitudinal strain, Left ventricular ejection fraction, anthracycline, CHOP chemotherapy

Background

Chemotherapy is described as one of therapies which are very effective in cancer treatment. One of the serious side-effects which have been noticed by Hematology and Cardiology experts is cardiotoxicity. One of the chemotherapy drugs that have cardio-toxic effects is anthracycline. This compound can cause arrhythmia, pericarditis, myocarditis and cardiomyopathy. The cardio-toxic effects of doxorubicin have been studied by various kinds of researches. The case of heart failure because of cardiotoxicity was first found in leukemia child patient in 1967 who underwent doxorubicin therapy1,2.

Non Hodgkin Lymphoma (NHL) is a primer malignancy which attacks lymphoid tissue and its supportive tissues, such as macrophages, histiocytes, and connective tissue. This disease comes from lymphocytes B, lymphocytes T and NK cells3. In 2010, it was presumed that there were 65,540 lymphoma cases that were diagnosed and caused 20,210 deaths4. In Indonesia, NHL was positioned on the sixth rank along with Hodgkin disease and leukemia as malignancy causes5. The number of malignant NHL cases in Indonesia since 1988 until 1991 was approximately 3.40% to 5.62% in males and, 79% to 3.40% in females6.

Doxorubicin in the Cyclophosphamide, Hydroxydoxorubicin, Oncovin, Prednisone (CHOP) regimen is still the golden standard of first-line chemotherapy in patients with NHL. It requires more attention even though the implementation of first-line chemotherapy will cause cardiotoxicity that will affect the prognosis of patients themselves7,8 doxorubicin, vincristin, prednisone. The case of acute cardiotoxicity after the
The cardiotoxicity causes myocardium damage and continues to decreased ventricular function progressively; then, it ends up being decomposition. The cohort research in 830 patients who underwent chemotherapy for 8.5 years was found 2.5% suffered from heart failure 10.

The risk of cardiotoxicity is caused by several factors such as old age, young age, female, drug consumption duration, mediastinal radiotherapy, history of heart disease, hypertension, and specifically cumulative dose of doxorubicin 11. The heart failure risks occur in 35% patients who obtain cumulative dose of 400 mg/m2, 7-26% in dose of 550 mg/m2, and 18-47% in dose of 700 mg/m2. The suggested administration of maximum cumulative dose is 400-500 mg/m2. The previous study reported that 27% patients who received doxorubicin with cumulative dose of ≥200 mg/m2 underwent cardiomyopathy although the contraction abnormality and ventricular relaxation have occurred in doxorubicin dose of ≤200 mg/m2 7,9,12 doxorubicin, vincristin, prednisone.

The early detection in cardiotoxicity and the preventive action of heart failure are considered as a challenge for cardiology and oncology experts. Several ways are conducted to monitor heart condition such as electrocardiography, echocardiography, radionuclide angiocardiography and endomyocardial biopsy as the golden standard 13,14.

Echocardiography is non-invasive examination that is received generally to evaluate the occurrence of cardiotoxicity15. This examination gives important information to recognize systolic heart function, especially left ventricular ejection fraction that is calculated by using teach method and modified simpson biplane 16. The previous study reported that the global longitudinal strain in 2D-STE has occurred in doses of 200 mg/m2 or the forth chemotherapy 17. This study aims to investigate the effect of CHOP chemotherapy implementation towards the GLS reduction in cumulative dosage of doxorubicin ≥ 100 mg/m2.

### Method

The study was a pre experimental study with modified cohort by using one group pretest posttest group design. It was conducted in Hematology-Medical Oncology Unit Dr. Soetomo General Hospital Surabaya.

The research subjects must fulfill the inclusion criteria which were clinical diagnosis of NHL and hispathology, and EF ≥60%. Subjects who have underwent chemotherapy before, had one of the diseases such as CKD, coronary heart disease, cardiomyopathy, and non-sinus EKG rhythm were excluded from study.

Patients was examined by echocardiography and taken the parameter of LVEF and GLS. Then, the NHL patients underwent CHOP chemotherapy until two cycles (doxorubicin cumulative doses of ≥100 mg). Patients who have undergone CHOP chemotherapy twice were examined by using echocardiography and taken the parameter of LVEF and GLS 1 week after. The echocardiography test was conducted with machine, namely Echo GE vivid 7 [GE Healthcare, USA]. The examination of 2D Speckle Tracking Echocardiography [2D-STE] was conducted with 2D and Automated Function Imaging by using imaging analysis software [EchoPac version 11O.x.x, GE Medical Systems, 2010]. The offline analysis was conducted in 2D imaging (apical long axis, apical 2 - and 4 – chamber) which was kept with frame rate approximately 50-70 bps. Each image was measured PSLS per segment and evenly calculated. Afterwards, the average value of the third value was automatically measured which was called Global Longitudinal Strain GLS. The LVEF test applied the Simpson’s biplane method.

The collected data in this study were analyzed descriptively and statistically. The statistical analysis to assess the different longitudinal strain parameter pre and post doxorubicin chemotherapy used paired t-test. The analysis to measure the correlation between decreased GLS and cardiotoxicity risk factors used Pearson correlation. The analysis of the data was conducted by using SPSS 17.0 program (SPSS, Inc., Chicago, IL) and it was significant if \( p \) value < 0.05.

### Results

#### Subject Characteristics

Most patients were males (73.3%). The average age of the subjects was 49±13 years old and the most frequent subjects based on age categorization were 61-70 years old with the total of 9 individuals (30%). The most frequent clinical stage was stage III which were 73.34% while the most frequent risk factor was smoking (50%; table 1).
**Table 1. Research Subject Characteristics**

<table>
<thead>
<tr>
<th>Variables</th>
<th>N [%] or average ±SB</th>
<th>Min-Max</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age [year]</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 11 – 20</td>
<td>1 [3.33]</td>
<td></td>
</tr>
<tr>
<td>- 21 – 30</td>
<td>1 [3.33]</td>
<td></td>
</tr>
<tr>
<td>- 31 – 40</td>
<td>7 [23.33]</td>
<td></td>
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<tr>
<td>- 41 – 50</td>
<td>8 [26.67]</td>
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<tr>
<td>- 51 – 60</td>
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<td><strong>Gender</strong></td>
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<tr>
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<td>- Females</td>
<td>8 [26.67]</td>
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<tr>
<td>- II</td>
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<td>- IV</td>
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<td>- Diabetes mellitus</td>
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<tr>
<td>- Smoking</td>
<td>15 [50.00]</td>
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</tbody>
</table>

**The Analysis Result**

The evaluation result of 30 NHL patients showed that there was no reduced EF more than 10%; however, it was obtained that 12 patients (40%) had reduced GLS > 10%, 10-15% in 7 patients (23.3%) and >15% in 5 patients (16.6%). The reduction of GLS >15% signified the occurrence of subclinical left ventricular dysfunctions (table 2).

**Table 2. The Analysis of EF and GLS before and after Chemotherapy**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Before</th>
<th>After</th>
<th>&lt;10%*</th>
<th>10-15%*</th>
<th>&gt;15%*</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEF [%]</td>
<td>65.5±3.1</td>
<td>63.9±2.8</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Global longitudinal strain [%]</td>
<td>-19.62±1.4</td>
<td>-18.03±1.9</td>
<td>18</td>
<td>7</td>
<td>5</td>
</tr>
</tbody>
</table>

Note: * Percentage of left ventricular systolic function decrease

**Discussion**

In this study, it was found that the administration of CHOP chemotherapy with a cumulative dose of 100 mg / m2 had an effect of decreasing GLS and causing subclinical left ventricular dysfunction. Swain et al. state that
LVEF is not an accurate predictor of heart failure on chemotherapy and prove there is no relationship between the severity of heart failure and the decrease in LVEF\textsuperscript{18}. The reverse results in the study of Nousiainen \textit{et al}. stated a decrease in LVEF 4\% after the cumulative dose of doxorubicin 200 mg / m\textsuperscript{2} has a sensitivity of 90\% and a specificity of 72\% as predictors of cardiotoxicity. In this study, 1 sample was found which experienced a LVEF decrease of 4\%\textsuperscript{19}.

In this study, there was no reduced LVEF >10\% after NHL patients underwent CHOP chemotherapy with the doses of ≥100 mg/m\textsuperscript{2}. On the other hand, the maximum reduced LVEF in this study was 4\%. This result was in accordance with the study of Stoodley \textit{et al}. in which with the doxorubicin doses of ≥ 200 mg/m\textsuperscript{2}, it has not been obtained reduced LVEF >10\%. However, Limat \textit{et al}. showed different result in which after the giving of doxorubicin cumulative doses of ≥ 200 mg/m\textsuperscript{2} or after the fourth chemotherapy in CHOP regimen, the occurrence of cardio-toxic was 27\% \textsuperscript{7,17,20} doxorubicin, vincristin, prednisone.

Chemotherapy Related Cardiac Dysfunction (CRCD) is divided into I and II. Anthracycline is considered as type I in which it can cause permanent and irreversible myocardium damage compared to type II that can cause reversible myocardium damage. Early detection is required to prevent myocardium cell damage widely and more severe. Swain \textit{et al}. stated that LVEF was not accurate predictor of heart failure in chemotherapy and proved that there was no correlation between the severity of heart failure and reduced LVEF. In contrast, Nousiainen \textit{et al}. asserted that reduced LVEF of 4\% after doxorubicin cumulative doses of 200 mg/m\textsuperscript{2} had the sensitivity of 90\% and specificity of 72\% as cardiotoxicity predictor. This study attained 1 sample that encountered reduced LVEF of 4\%\textsuperscript{12,18,19,21}.

Several previous studies have showed the superiority of GLS test compared to LVEF test. Most population that was being analyzed using GLS parameter was breast cancer by employing the combination regimen of anthracycline and trastuzumab. Researches which employed GLS and NHL as their parameter are rarely conducted. Kang \textit{et al}. used GLS parameter and high sensitivity of troponin-T as cardiotoxicity predictor in NHL. GLS monitoring is conducted on baseline, 1 day after the third cycle, and 1 day after completing chemotherapy. The GLS monitoring in this study was conducted on baseline, and 1 week after the second cycle\textsuperscript{22}.

GLS test is considered capable in detecting subclinical cardio-toxic and has good reproducibility. Expert Consensus Statement recommends GLS test to detect subclinical left ventricular dysfunctions rather than Global Radial Strain [GRS] and Global Circumferential Strain [GCS] because there has not been normal value and different variability between vendor and software. The abnormal reduced GLS value is different in several studies. Stoodley \textit{et al}. determined abnormal reduced GLS value of >10\%\textsuperscript{17}, Negishi \textit{et al}. determined reduced GLS of >11\%\textsuperscript{23}. Banchs suggested that reduced GLS of >10\% was considered in giving anti-remodeling therapy\textsuperscript{24} and clinical monitoring strictly together with oncologist whereas Kang \textit{et al}. determined reduced GLS of >15.9\%\textsuperscript{22}. The abnormal GLS value in this study based on Expert Consensus Statement ASE that stated reduced GLS of >15\% from baseline showed that subclinical left ventricular dysfunctions\textsuperscript{17,21–25}. In this study there was no correlation between reduced GLS and hypertension risk factors while there was no correlation between reduced GLS and other factors such as age, diabetes, and smoking. It was in accordance with the study conducted by Hershman \textit{et al}. in which only hypertension risk factor that synergized with cardio-toxic cases\textsuperscript{26} which improves survival for patients with non-Hodgkin’s lymphoma, is often withheld from elderly patients because of its cardiotoxicity. We studied the cardiac effects of doxorubicin in a population-based sample of older patients with diffuse large B-cell lymphoma (DLBCL).

**Conclusion**

There was an effect in the implementation of CHOP chemotherapy towards global longitudinal strain. The giving of doxorubicin cumulative doses of ≥ 100 mg/m\textsuperscript{2} was able to cause subclinical left ventricular dysfunctions.

**Ethical Clearance:** The study protocol was approved by the Ethical Commission to conduct basic science/clinical research in Dr. Soetomo General Hospital Surabaya, Indonesia. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, non-maleficence, and justice.

**Conflict of Interest:** The author reports no conflict of interest of this work.
**Source of Funding:** This study is done with individual funding.

**References**


Effect of Metronidazole on Clopidogrel Bisulphate Efficacy in Male Albino Rats

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²Department of Pharmacology and Toxicology, Assistant lecturer, College of Pharmacy- University of Anbar,
³Department of Pharmaceutical Chemistry, Assistant lecturer, College of Pharmacy, University of Anbar

Abstract

The data about this combination effect on platelet function and hematological parameters are sparse, but there is only mention in many medical science websites such as Medscape, there are possible interaction between it, in which metronidazole cause inhibition to cytochrome CYP3A4 which responsible on clopidogrel metabolism, this may put the patients that on clopidogrel bisulphate therapy at risk of clot formation and may be progress, this may occur because clopidogrel is at inactive form and need to activation by cytochrome mention above, so this study was performed in order to achieve the following aims: studying the efficacy of clopidogrel bisulphate on coagulation process by measuring bleeding time when giving clopidogrel alone and in combination with metronidazole and studying the possible effect of two drug alone and in combination on hematological parameters in male albino rats.

Keywords: Clopidogrel bisulphate, metronidazole, cytochrome CYP3A4, bleeding time

Introduction

Clopidogrel, a thienopyridine derivative, binds precisely also permanently to the platelet P2RY12 purinergic receptor, constraining ADP-mediated platelet motivation and aggregation (¹). Next oral taking of clopidogrel is promptly absorbed owned to more metabolism, clopidogrel is one of prodrug that is absorbed in the intestine and activated in the liver, then it converted to its active metabolite needs dual sequential oxidative steps, at the first time result in formation of 2-oxo-clopidogrel, followed by the conversion of 2-oxoclopidogrel to the active metabolite. CYP2C9 and CYP3A4/5 are connected to cytochrome P450 enzymes involved in the metabolism of clopidogrel (²). CYP2C9 is most important hepatic enzymes involved in the formation of clopidogrel’s active metabolite. Genetic polymorphisms of CYP2C9 are associated with impaired clopidogrel metabolism in healthy volunteers and also in patients (³). Metronidazole is antibiotic and antiprotozoal medication. it is one type for a first event of mild-to-moderate Clostridium difficile colitis if vancomycin or fidaxomicin is unavailable (⁴). It has been suggested possible inhibition of cytochrome P450 especially CYP2C9 and CY3A4, this enzyme is responsible for several drugs metabolism such as S-warfarin etc.⁵. And this may appear in revers effect when combined with clopidogrel when compared with warfarin because the difference between two drugs chemically (warfarin is active drug and this combination cause abnormal bleeding).

Materials and Method

Experimental animals: A total number of twenty male Albino Wistar rats weighed (200-250 g) were utilized in this research. Their ages ranged between 4-5 months. Rats in all time of the experiment were housed in plastic cages in conditioned room (22-25°C) in the animal house of College of pharmacy – University Of Anbar with manually controlled lightening supplying daily light of 12 hours (7.00 AM to 19.00 PM) and 12 hours night cycle. They were left for 7 days for adaptation with the research conditions. Rats had free access to water and diet along the experimental period.

Experimental Design: Rats were randomly divided into four equal groups (5rat/group) and administered orally for 28 days by using special gavage needle as the following: C group rats that received ordinary distilled water and served as control, T1 group rats were treated...
1.25 mg/kg B.W of clopidogrel bisulphate, T2 group Rats were treated 35 mg/kg B.W of metronidazole and T3 group rats were treated 1.25 mg/kg B.W of clopidogrel bisulphate + 35mg/kg B.W of metronidazole.

**Bleeding time test performance:** Bleeding time was measured via rat tail transection method according to the prescription of The tail of the rat was warmed for 60 seconds in water at 40°C then dried (6). A slight cut was made in the mid of the tail with a blade. Account started when the 1st drop touched the circular filter paper. It was tested at 30 s intervals till bleeding cosseted

*Total number of spot on filter paper/2= BT/minute.

**Hematological Analysis:** All animals in experiment comparing with the normal values of blood parameters as control and zero time. The blood collected from heart puncture after general anesthesia by using (Ketamine and xylazine). By using Uto-analyzer (setting on vet mode) Platelet count, RBC, WBC, and HB level were estimated. The procedure done according to manufactured instruction.

**Statistical Analysis:** The data was accomplished by using SAS (Statistical Analysis System - version 9.1). One-way ANOVA, two-way ANOVA and Least significant differences (LSD) post hoc test were complete to assess significant differences among means. P < 0.05 was considered statistically significant.

**Results**

Before beginning of dosage of experiment (at zero time), Bleeding time and other hematological parameters was determined and there was no significance different (P <0.05) in all groups.

1. **Bleeding time:** A statistical analysis indicated that the mean value of bleeding time was non-significantly (P<0.05) differed in all experimental groups at zero time when compared to each other. The final results showed significant (P>0.05) increased after oral intubation of clopidogrel bisulphate, metronidazole and combination in T1, T2 and T3 groups comparing to the control group and zero time. The T1 group that receive 75 mg/Kg/day of clopidogrel bisulphate was more potent in increasing of bleeding time comparing to 35 mg/kg/day of metronidazole and in combination treated group (T2, T3) respectively.

<table>
<thead>
<tr>
<th>Group</th>
<th>Day of experiment</th>
<th>Zero time</th>
<th>End of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>A2.40±0.18a</td>
<td>A2.30±0.12d</td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>B2.50±0.22a</td>
<td>A13.90±0.18a</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>B2.60±0.18a</td>
<td>A3.80±0.12c</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>B2.50±0.15a</td>
<td>A7.20±0.12b</td>
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</tr>
<tr>
<td>LSD</td>
<td>0.4831</td>
<td></td>
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</tr>
</tbody>
</table>

- Different Capital letters denote differences between groups, (P<0.05).

2. **Platelet count:** The effect of oral intubation of 75 mg/Kg/day of clopidogrel bisulphate (T1), 35 mg/Kg/day metronidazole (T2) and combination dose of both of them T3 for 28 day on treated rats RBCs count are clarified in table 2. There were none considerable (P<0.05) differences in platelet count between empirical groups in zero time, at the finish of research there was a significant (P<0.05) reduction in platelet count in T1 and T3 groups respectively compared with control group, zero time and T2 group. A high significant decreases (P<0.05) were recorded in animal of T1 group followed by T3 (group of combination treatment).

<table>
<thead>
<tr>
<th>Group</th>
<th>Day of experiment</th>
<th>Zero time</th>
<th>End of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>A200.40±0.50ab</td>
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<tr>
<td>T1</td>
<td>A201.80±0.58a</td>
<td>C178.00±0.44c</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>A201.20±0.86a</td>
<td>A200.20±0.37a</td>
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</tr>
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<td>T3</td>
<td>A199.80±0.37b</td>
<td>B186.80±0.80b</td>
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<td>LSD</td>
<td>1.6797</td>
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- Different Capital letters denote differences between groups, (P<0.05).

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Table 1: Effect of oral intubation for 28 days with Clopidogrel bisulphat, metronidazole and their combination on bleeding time (by tail method as prescribed previously) of treated adult male rats. (min)

Table 2: Effect of oral intubation for 28 days with Clopidogrel bisulphat, metronidazole and their combination on platelet count of treated adult male rats (X10^9/μl).
- Different Small letters denote differences within group, *(P< 0.05).*

3. **Red blood cells count:** A statistical analysis indicated that the mean value of RBCs count was non-significantly *(P<0.05)* differed in all experimental groups at zero time when compared to each other. At the end of experimental RBCs count was significantly *(P>0.05)* decreased after oral intubation of clopidogrel bisulphate, metronidazole and combination (clopidogrel bisulphate plus metronidazole) in T1, T2 and T3 groups comparing to the control group. The extent of decrement was higher in T1 group, where as in T3 group (combination therapy) the change was at little degree when compared with T1 group.

**Table 3:** Effect of oral intubation for 28 days with Clopidogrel bisulphat, metronidazole and their combination on RBC count of treated adult male rats *(X10^6/µl).*

<table>
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<td>A8.44±0.01a</td>
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<td>T1</td>
<td>A8.33±0.09a</td>
<td>C5.26±0.04c</td>
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<tr>
<td>T2</td>
<td>A8.35±0.09a</td>
<td>A7.26±0.04b</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>A8.26±0.10a</td>
<td>B6.09±0.02c</td>
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<tr>
<td>LSD</td>
<td>0.6602</td>
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- Different Capital letters denote differences between groups, *(P<0.05).*
- Different Small letters denote differences within group, *(P< 0.05).*

4. **WBC count:** Table 4 illustrates the mean value of WBC count in the control and treated group throughout the experimental period. It can be seen that WBC count were in the normal in all groups at the zero time, after animals received clopidogrel bisulphate, metronidazole and combination of them a significant fall *(p<0.05)* in WBC count, a significant *(P<0.05)* decrease in WBC count was observed in all treated groups (T1, T2, T3) comparing to the pretreated period but at a different extent more potent in T1 followed by T3 and T2 group.

**Table 4:** Effect of oral intubation for 28 days with Clopidogrel bisulphat, metronidazole and their combination on WBC count of treated adult male rats *(X10^3/µl).*

<table>
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<th>Day of experiment</th>
<th>Zero time</th>
<th>End of experiment</th>
</tr>
</thead>
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</tr>
<tr>
<td>T1</td>
<td>A9.53±0.07a</td>
<td>D6.10±0.03d</td>
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<td>T2</td>
<td>A9.55±0.12a</td>
<td>B8.31±0.05b</td>
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</tr>
<tr>
<td>T3</td>
<td>A9.51±0.06a</td>
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<tr>
<td>LSD</td>
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</table>

- Different Capital letters denote differences between groups, *(P<0.05).*
- Different Small letters denote differences within group, *(P< 0.05).*

5. **Hemoglobin concentration:** Table 5 illustrates the mean value for Hb concentration in the control and treated group throughout the experimental period. It can be seen that hemoglobin concentrations were in the normal in all groups at the zero time, after animals received clopidogrel bisulphate, metronidazole and combination of them a considerable fall *(p<0.05)* in hemoglobin concentration after 28 days of the treatment as compared with pretreated period and control group, this decrease is coincided with the decrease of RBCs in all groups of experiment.

**Table 5:** Effect of oral intubation for 28 days with Clopidogrel bisulphat, metronidazole and their combination on Hemoglobin concentration of treated adult male rats *(mg/dl).*

<table>
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<td>T1</td>
<td>A15.05±0.11a</td>
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<tr>
<td>T2</td>
<td>A14.84±0.12a</td>
<td>B13.59±0.05d</td>
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</tr>
<tr>
<td>T3</td>
<td>A15.03±0.15a</td>
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<tr>
<td>LSD</td>
<td>0.3522</td>
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<td></td>
</tr>
</tbody>
</table>

- Different Capital letters denote differences between groups, *(P<0.05).*
Different Small letters denote differences within group, (P< 0.05).

Discussion

It was reported that clopidogrel bisulphate cause a significant change in bleeding time in 36 human volunteer after giving a 75 mg of Plavix (7). Also another study indicated that clopidogrel bisulphate associated with significantly increased risk for postoperative wound complications especially increasing of bleeding time in tested patient (8). These normally cause increasing of bleeding in T1 group. In T2 group treated with metronidazole alone, it was reported a partial inhibition platelet aggregation with dose and time dependent in guinea pig and human platelet-rich plasma and this may cause a mild increasing in bleeding time according to dose that we give in comparison with previous study (9).

In T3 the combination of clopidogrel bisulphate and metronidazole which sharing same metabolic enzyme or cytochrome that may lead to inhibition or slowing the process of clopidogrel bisulphate converting to its active form (10), this mention why the combination of two drugs causing less bleeding time than clopidogrel bisulphate alone treated group. Many of case report indicates that in addition to the increased risk of bleeding with the use of Clopidogrel, some of its additional serious side effects include agranulocytosis, aplastic anemia/pancytopenia, thrombotic thrombocytopenic purpura, acquired hemophilia A, and neutropenia, neutrophils make up

Figure (1): showed the steps of experiment performance starting from housing of animals (A), dosing of the drugs (B), (C) anesthetizing animals, (D) bleeding time test, and (E&F) blood collection for hematological test parameters.
a significant portion of the WBCs (11). With regard to metronidazole there was reported that metronidazole can cause bone marrow depression (12). And normally this can affect all type of blood cells especially in chronic use

**Conclusion**

The efficacy of clopidogrel bisulphate as blood thinner agent may be reduced when combined with metronidazole despite of the second have characteristic of antiplatelet agent, this were obvious in bleeding time test results and other hematological parameters, may be due to the inhibition of metronidazole to cytochrome that responsible for clopidogrel metabolism, in addition to possibility of compete the two drugs and/or inhibition of each other. This still need to more clearing by check the platelet activity in presence of this combination by more specific tests and apparatus. more attention should be paid when use the metronidazole in patient who take clopidogrel bisulphate especially critical cases by putting the patient under close monitoring or use alternative drugs.

**Conflict of Interest:** personal relationship

**Source of Funding:** self

**Ethical Clearance:** institutional

**References**

The Influence of Slow Back Stroke Massage, Cold-compress and Warm-compress to the Level of Prostaglandin F2α (PGF2α) in Primary Dysmenorrhea

Mukhoirotin¹, Kurniawati¹, Diah Ayu Fatmawati¹
¹Nursing Science Program, Faculty of Health Science, Unipdu Jombang

Abstract

The cause of primary dysmenorrhea is excessive release of the hormone prostaglandin F2-alpha (PGF2α). The aim of this study was to assess the influence of SBBM, cold-compresses and warm-compresses toward the level of PGF2α in primary dysmenorrhea, using Post Test Only with Control Group Design and Pretest-Postest Control Group Design. The subjects were 76 female-student, selected by purposive sampling. NRS used to measure the pain level and ELISA used to measure PGF2α levels. The data were analyzed by using Paired Sample T-Test, One-Way Anova and Kruskal-Wallis test. SSBM, cold-compresses and warm-compresses are effective to decreasing of pain level and PGF2α levels in primary dysmenorrhea.

Keywords: Slow Stroke Back Massage, Cold-compresses, Warm-compresses, PGF2α, menstrual pain

Introduction

This incidence of dysmenorrhea is 20% to 90% among reproductive women and 15% occurred among young female followed with pain level around 2%-29%. Primary dysmenorrhea commonly occurred among adolescents without any pathological problems at the pelvis. The prevalence of primary dysmenorrhea among adolescent was 55.5-90.1%. In Mexico, dysmenorrhea affects 65% of students’ daily activities.

The cause of primary dysmenorrhea is an increase in prostaglandin F2-alpha (PGF2α) production. The treatment of dysmenorrhea commonly used nonsteroidal anti-inflammatory drugs (NSAIDs). As we know medication consumption for long time will give some impact such as medication addictive, diarrhea, abdominal pain, nausea, kidney-complications, liver-complications, sleep-disorders, digestion-problems. The pharmacological treatment for dysmenorrhea was successful, but about 20-25% of the failure process. The one of the traditional treatment for dysmenorrhea is Slow Stroke Back Massage (SSBM), cold-compresses and warm-compresses. SSBM reduces pain and effectively increases endorphin levels. Cold-compresses reduce prostaglandins, so it will make strengthens the sensitivity of pain and other subcutaneous at the injury site by inhibiting the inflammatory process and giving analgesic. The skin stimulation causes endorphin loose so it will block blocking the transmission of pain stimulus. Skin stimulation activates the transmission of sensory A-Beta nerve fibers and decreases pain transmission C and delta-A fibers so the synaptic gate closes the transmission of pain impulse. Cold-compresses and warm-compresses are stimulates to loosing the endorphin β levels and regulate uterine hypercontractility during menstrual pain.

The results of previous studies indicated that Moxibustion consumption can reduce the levels of PGF2α, OT, vWF and increase the levels of β-EP (β Endorphin). However, the influence of SSBM, Cold-compresses and Warm-compresses toward the intensity of pain and PGF2α levels in Primary Dysmenorrhea have not been clearly known. The aim of this study was to analysis the effect of SSBM, Cold-compresses and Warm-compresses toward Pain Intensity and Prostaglandin F2α levels in Primary Dysmenorrhea.

Method

The design in this study was Post Test Only with Control Group Design and Pretest-Postest Control Group Design. The sample was 76 female students in faculty of health science of Unipdu Jombang who experienced dysmenorrhea, selected by purposive sampling. The samples were divided into six groups: SSBM (n=13), cold-compress (n=12), warm-compress (n=13), SSBM+cold-compresses (n=13), SSBM+warm-compresses (n=13), control (n=12). The data were collected by two istruments: NRS (Numeric Rating Scale) and ELISA (Enzyme-Linked Immunosorbent Assay) to measure PGF2α levels.
Scale) to measured the pain level, ELISA to measured PGF2α. Data were analyzed by Paired Sample T-Test, One-Way Anova and Kruskal-Wallis test.

**FINDINGS**

**Table 1. Intensity of menstrual pain before giving intervention**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSBM</td>
<td>6.31</td>
<td></td>
</tr>
<tr>
<td>Cold-compress</td>
<td>6.75</td>
<td></td>
</tr>
<tr>
<td>Warm-compress</td>
<td>6.31</td>
<td></td>
</tr>
<tr>
<td>SSBM+cold-compress</td>
<td>6.15</td>
<td>0.747</td>
</tr>
<tr>
<td>SSBM+warm-compress</td>
<td>6.77</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.17</td>
<td></td>
</tr>
</tbody>
</table>

One-Way Anova, Post Hoc Tamhane’s test

**Table 2. The menstrual pain after giving intervention**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSBM</td>
<td>3.54</td>
<td></td>
</tr>
<tr>
<td>Cold-compress</td>
<td>2.83</td>
<td></td>
</tr>
<tr>
<td>Warm-compress</td>
<td>2.85</td>
<td></td>
</tr>
<tr>
<td>SSBM+cold-compress</td>
<td>2.23</td>
<td>0.000</td>
</tr>
<tr>
<td>SSBM+warm-compress</td>
<td>3.08</td>
<td></td>
</tr>
<tr>
<td>Positive-control</td>
<td>6.00</td>
<td></td>
</tr>
</tbody>
</table>

One-Way Anova test, Post Hoc Tamhane’s test

**Table 3. The differences of menstruation pain between before and after intervention group**

<table>
<thead>
<tr>
<th>Group</th>
<th>Before Mean±SD</th>
<th>After Mean±SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSBM</td>
<td>6.31±0.95</td>
<td>3.54±0.88</td>
<td>0.000</td>
</tr>
<tr>
<td>Cold-compress</td>
<td>6.75±1.29</td>
<td>2.83±1.19</td>
<td>0.000</td>
</tr>
<tr>
<td>Warm-compress</td>
<td>6.31±1.18</td>
<td>2.85±0.89</td>
<td>0.000</td>
</tr>
<tr>
<td>SSBM+cold-compress</td>
<td>6.15±1.28</td>
<td>2.23±0.09</td>
<td>0.000</td>
</tr>
<tr>
<td>SSBM+warm-compress</td>
<td>6.77±1.83</td>
<td>3.08±1.80</td>
<td>0.000</td>
</tr>
<tr>
<td>Control</td>
<td>6.17±1.40</td>
<td>6.00±1.21</td>
<td>0.166</td>
</tr>
</tbody>
</table>

Paired Sample T-Test

**Table 4. The differences of PGF2α level between intervention group and control group**

<table>
<thead>
<tr>
<th>Group</th>
<th>Median pg/ml</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSBM</td>
<td>132.967</td>
<td></td>
</tr>
<tr>
<td>Cold-compress</td>
<td>136.033</td>
<td></td>
</tr>
<tr>
<td>Warm-compress</td>
<td>145.367</td>
<td></td>
</tr>
<tr>
<td>SSBM+cold-compress</td>
<td>151.300</td>
<td></td>
</tr>
<tr>
<td>SSBM+warm-compress</td>
<td>136.767</td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>156.544</td>
<td>0.017</td>
</tr>
</tbody>
</table>

Kruskal-Wallis test, Post Hoc Mann-Whitney test.

**Discussion**

The menstrual pain level before give the interventions were moderate to severe and after give treatments the average menstrual pain level in all treatment groups was mild and in control group was moderate level. The intensity of menstrual pain in this study was addressed with some previous studies which showed that the menstrual pain level among he respondents was moderate to severe level(19-20). Some risk factors of dysmenorrhea are include the early of age menarche, the longer of menstrual periods, the higher of menstrual volume, family history of dysmenorrhea, smokers, obesity, alcohol consumption(2,21-22), high caffeine diet(23), and high levels of stress(24).

The results of this study showed that the average of menstrual pain level in all intervention groups are significantly decrease between pre-test and post-test. This showed that SSBM, cold-compresses and warm-compresses, SSBM+cold-compresses, SSBM+warm-compresses are effective to reduce the menstrual pain level. These interventions are cutaneous stimulation techniques, where the mechanism of these interventions in reducing pain level was explained according to the gate control theory. Gate control theory explained that skin stimulation can activate the transmission of the fibers of A-Beta nerve sensory(25). This process can reduce pain transmission through small diameter of C and delta-A fibers so the synaptic gate will closes the transmission of pain impulse. Generally, the cutaneous stimulation on the body is on the back and shoulders. Cutaneous stimulation are influenced peripheral fibers to send the impulses by using dorsal horn in the spinal cord, when the impulses carried by A-Beta fibers are dominate so the gate mechanism will close so the pain...
impulses are not delivered to the brain\(^{(26)}\).

The average decrease of menstrual pain level in SSBM-group. Touch and massage are the technique of sensory integration that affect the activity of nervous system. Individuals who perceived touch as a stimulation to relax, a relaxation response will appear. The use of appropriate cutaneous stimulation can reduce pain perception and muscle tension. Conversely muscle tension can increase pain perception\(^{(16)}\). Massage of connective tissue makes relaxes on body, reduces muscle spasms, connective tissue tenderness, increases the circulation and \(\beta\)-endorphin plasma\(^{(27)}\). This intervention can stimulate the autonomic nervous system to balance the sympathetic and parasympathetic nerves\(^{(28)}\). The SSBM was effective in reducing menstrual pain level\(^{(14)}\). The connective tissue manipulation (CTM) can be used as an intervention to primary dysmenorrhoea and menstrual-related symptoms because this method didn’t give the potential effects such as analgesic, noninvasive and easy to do\(^{(29)}\).

The average of menstrual pain level in cold-compress group was ranged 6.75-2.83. The intervention of cold-compress in the abdomen for 20 minutes by using a bag filled ice. The intervention of cold-compress to respondents was helpful to reduce the pain level, this because of the blood flow was decreased in area which is compressed, and gave analgesic effect by slowing the speed of nerve delivery so the pain impulses will be decrease or less to the brain. Cold-compresses was gave the physiological effects to reduce the inflammatory response, reduce blood flow, reduce edema, and reduce local pain\(^{(20)}\). Cold therapy (ice compresses) intervention will be blocked and the impulses of pain will be reduce or loose for few times\(^{(26)}\). Previous research results showed that cold-compresses was effective reduced menstrual pain\(^{(31)}\).

After given of warm-compresses the average of menstrual pain significantly decreased. Warm-compresses is an intervention that can help to reduce pain by using dilated method of blood vessels so this will increasing the blood supply to the body\(^{(32)}\). Some previous research said that warm-compresses was effected to reduce menstrual pain level\(^{(33-34)}\).

In the control group after given management information about menstrual pain, a small proportion experienced a decreasing of menstrual pain level but was not significant. The information was included pharmacological and non-pharmacological menstrual pain treatment. Non-pharmacological treatment are regular exercise, adequate rest, warm-compresses on abdomen, yoga, warm water shower, massage, deep breath, acupunctur, acupressure, TENS, salt and sugar diet, cold-compresses and deuretics consumption (asparagus and watermelon). Pharmacological treatments are hormonal-drugs and NSAIDs\(^{(35)}\).

The results of this study showed that there were no significant differences in PGF2\(\alpha\) levels in all intervention groups, there were significant differences in PGF2\(\alpha\) levels between intervention and control. Primary dysmenorrhoea caused by the increasing of prostaglandin production and the release of endometrial prostaglandins during menstruation so it will induce uterine hypercontractility, reduce uterine blood flow, and hypersensitive of pain\(^{(36)}\). Menstrual cramps more worse because PGF2\(\alpha\) was increased and PGE2 decreases, so primary dysmenorrhoea increased the ratio of PGF2\(\alpha\) to PGE2\(^{(37-38)}\).

Massage is a therapy to reduce the production of NF-\(\kappa\)B, inflammatory cytokines and TNF-\(\alpha\)\(^{(39)}\). Inflammatory cytokines (ex: IL1-\(\beta\), TNF-\(\alpha\)) is stimulates prostaglandin production in the first day of menstrual phase\(^{(40-41)}\). The results of previous studies indicated that the Swedish Massage Therapy reduced the level of Mitogen-Stimulation of IL-1\(\beta\), IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, and IFN-g\(^{(42)}\). The decreasing of inflammatory cytokines impacted decreasing of prostaglandin stimulation production. Therefore, the SSBM intervention can reduce prostaglandin levels in primary dysmenorrhoea.

Cold-compresses are the intervention which give vasoconstriction effects, prevented the edema, reduce inflammation, local anesthetics effect, reduce cell metabolism and increase blood viscosity. Warm-compresses are the interventions which give a vasodilation effects to blood circulation to becomes smooth and muscles relax\(^{(32)}\). Hot stimulation can increase blood flow in blood vessels at uterus and consequently the prostaglandin vascular will be liquid, bradykinin, and histamine\(^{(25)}\). Heat interventions in local area is as effective as NSAIDs\(^{(43)}\). The NSAIDs mechanism to relieve primary dysmenorrhoea depends on the inhibition of cyclooxygenase (COX), an enzyme which responsible for the prostaglandins production and (other prostanooids). The unbalanced amount of prostaglandins from the endometrium during menstruation are major cause of dysrhythmias.
contraction at the uterus, the reducing of local blood flow and the increasing of peripheral nerve sensitivity during Primary Dysmenorrhea. Therefore, cold-compresses and warm-compresses are same effectively to reducing prostaglandins.

Conclusion

SSBM, cold-compresses and warm-compresses are effectively reduced pain level and PGF2α levels to dysmenorrhea.

Ethical Clearance- obtained from the ethics commission of Nursing Faculty of Airlangga University, Surabaya.

Conflict of Interest- No

Source of Funding-Directorate of Research and Community Service, Directorate General of Research and Development Strengthening, Ministry of Research, Technology and Higher Education.

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Enhanced the Antibacterial and Mechanical properties of UHMWPE by Addition Sort Fibers of Polyacrylonitrile PAN, Graphene Nanoplate (GNP) and Hydroxyapatite (HAp)

Najim A. Saad¹, Massar Najim Obaid¹

¹ College of Materials Engineering – University of Babylon, Iraq

Abstract

The UHMWPE is a gold material in total joint replacement due to excellent properties but with the time was degradation therefor, In present study it was reinforced with 5% short fibers of polyacrylonitrile (PAN) and different weight fraction (0.3, 0.8 and 1.3)% of graphene nanoplate (GNP) and hydroxyapatite (HAp) to increase the mechanical properties (flexural strength, flexural modulus, compression strength and hardness) due to good bonding between fibers and nanoparticles (GNP, HAp) with polymer which improved the load transfer from matrix to reinforcement phases, and also attributed to good mechanical properties of reinforcement fillers. Moreover, these fillers maintained the antibacterial properties to avoid infection which can happened per implantation of knee joint replacement.

Keyword: UHMWPE; PAN; GNP; HAp; Antibacterial Properties and Flexural Strength.

Introduction

Ultra high molecular weight polyethylene, UHMWPE have been used as bearing components in total joint replacements due to their excellent properties. Such as low friction coefficient against metallic components, high impact strength, and good chemical resistance, was widely investigated as a biomaterial especially as a bearing material in the total joint prostheses [1]

The great success of surgically-implanted biomaterials may be compromised in every case by the challenging complication of bacterial per implant infection [2]. Approximately 2.6 million orthopedic biomaterials are implanted annually in the USA, hence the incidence of implant-associated infections is also increasing [3]. Most important in the pathogenesis of infection is the colonization of the device surface and the consecutive formation of a biofilm [4], in which Staphylococcus aureus and koagulase –negative Staphylococci are most frequently implicated as the etiologic agents [5]. Systemic antibiotic prophylaxis and various local antibiotic delivery techniques have been proven to reduce the rate of infection [6]

Polyacrylonitrile PAN hollow fibers are already used as dialyzers that eliminate proteins and low molecular weight compounds. PAN fibers have high surface area, very high mechanical strength, abrasion resistance and had insect resistance. However, PAN has various greater properties, the existence of nitrile groups along with the fiber backbone deals multidirectional methods to alter fibers for definite applications dissimilar artificial films which can be damaged during the alteration [7].

Graphene is a one-atom-thick sheet of carbon atoms arranged in a 2D honeycomb structure. The strong carbon-carbon bonding in the plane, the aromatic structure, the presence of free p electrons, and reactive sites for surface reactions make graphene a unique material with excellent properties [8].

The polymer composites reinforced with graphene had better elastic modulus, toughness, hardness and fatigue strength. The main benefits of graphene as reinforce phase improved load transfer from a matrix to reinforcement due to have excellent in-plane strength and actual high surface area. [9,10]

Nano-hydroxyapatite who the main mineral constituent of bone, is one of the greatest interesting inorganic materials for applications in bone re-forming, thus it has been broadly used in hard tissue engineering to stimulate biological properties of bio-inert polymer by compound process. [11]
Hydroxyapatite-reinforced polymer composites display attractive properties for biomedical uses because of the existence of HA increases the biological properties of the material [12].

In current study, the hybrid nanocomposite of UHMWPE reinforced by 5% PAN with different weight fraction of nanoparticles (GNP, HAp) were prepared and examined mechanical properties (flexural and compression strength) and anti-bacterial properties for prepared hybrid nanocomposites to use in knee joint replacement.

**Experimental Procedure**

Ultra high molecular weight polyethylene (UHMWPE) was gained as a powder from LUOYANG MAX PIPE INDUSTRY with Molecular weight 600-700×10^4 g/mol., density (0.093-0.94) g/cm^3 and Granularity (≥99%) 20-40 Mesh.

Polyacrylonitrile (PAN) is a short fiber was obtained from TENGZHOU TUOLDUO INDUSTRIAL & TRADE CO., LTD, with length 3mm, diameter (15 ± 2) µm and density 1.18±0.01 g/cm^3.

Graphene nanoplate are two dimension Nanoparticles obtained from TIANRUN SUNSHINEGRAPHITE CO., LTD with purity >95wt.% , Thickness (1.0-1.7) nm, diameter (0.5-5) µm, (2-5) layers and SSA (360-450 m^2/g).

Hydroxyapatite (HAp 04) is a nanopowder were used in this research are acquired from N&R INDUSTRIEIES, INC with purity 99% and Average Particle Size (20nm).

The two groups of hybrid composite material were prepared by addition 5% PAN short fibers and addition different weight fraction (0, 0.3, 0.8 and 1.3%) of graphene nanoplate GNP for first group and hydroxyapatite for second group. the nanoparticals are dispersion in ethanol by sonication instrument for 30 min for HAP and 15 min for GNP after that the nanoparticles were mixing by mechanical mixing the PAN fibers are added gradually and also dispersion in the sonication instrument for 15 min, the mixture are mixing by mechanical mixing and the powder of UHMWPE are added gradually. the mixing process were continued for 30 min. After that, the mixture was put in an oven with vacuum for drying at (60°C). After that the mixture was put in mold and press in hydraulic press at temperature 180 °C and pressure 12 MPa for one hour. Then the mold left to cool in air up to room temperature to get the sheet of composite material which was cut by CNC laser machine according to ASTM standard of each test in present study.

The samples of flexural properties test are cut from sheet according to ASTM D790 [13], the sample dimensions (100×10×4) mm. the test (three point - type) is achieved after fixed the ends of sample the loud applied at midpoint at strain rate 5mm/min. also The five sample are tested each time.

The samples of compression strength test are cut from sheet according to ASTM D695-02a. the test is achieved at velocity 1.3mm/min and the loud applied until the sample is failed. The data of stress-strain are obtained. each time The five sample are tested and the mechanical properties are the average of the data of sample [14].

Hardness test is required to measure the resistance of material to indentation, shore D instrument model (TH 210 FJ) made in Germany, the specimen is Cylindrical shape, the test was being done according to ASTM (D2240) [15]. To obtained correct readings the surface of sample must be smooth and clean and the minimum thickness of sample is 4mm, the position at which the test is proceed is far at least from the edge is 12mm. Each specimen was tested five times at different positions and the final hardness is average of them.

By using the Agar Well Diffusion Method was being used to determinate antibacterial activity of the samples. the Muller Hinton agar plates were prepared and were inoculated with Escherichia coli (E. coli), staphylococcus aureus (S. aureus) as test organisms which spreading on the surface of the media with the help of sterile swab.

Neat Polymer and polymer nanocomposites samples have been inserted to the plates at two state (solid after press, liquid state before evaporate the ethanol and also pure ethanol) to determine the inhibition zone. Finally, these plates were incubated at 37°C, and after 24 hours of incubation; zones of inhibition were visualized and measured the diameter of the inhibition zone and recorded in mm.

**Results and Discussion**

Figure (1) described the relationship between the flexural strength with different weight fraction.
of nanoparticles (GNP and HAp) w.t %, the figure illustrated the flexural strength was increased with addition PAN short fibers and increased with increased weight fraction of HAp% and also increased with weight fraction of GNP% up to 0.8% due to good interfacial bonding between matrix and reinforcement phases (PAN fibers and nanoparticles) thus impeded the growth of cracks within polymer, but at 1.3% GNP slightly decreased due to agglomeration of GNP. The flexural strength was increased from (16.2) for neat UHMWPE, (20.4075 MPa) for (UHMWPE + 5% PAN) to (28.317 MPa) for (UHMWPE + 5% PAN + 0.8% GNP) and to (27.228 MPa) for (UHMWPE + 5% PAN + 1.3% HAp). Generally, the increment in flexural strength at addition GNP was higher than at addition of HAp [16-18]

Figure (1) The Flexural Strength of Nanocomposite materials as function of Nanoparticles wt.% content in Nanocomposite

Figure (2) described the relationship of flexural modulus with weight fraction of nanoparticles (GNP + HAp) %. This figure showed the flexural modulus increased with addition of PAN fibers and with increased weight fraction of HAp%, also increased with increased weight fraction of GNP% up to 0.8% due to the properties of nanoparticles which had flexural modulus higher than UHMWPE and also due to good interfacial bonding between matrix and reinforcement phases (PAN fibers and nanoparticles) [16]

Figure (2) The Flexural Modulus of Nanocomposite materials as function of Nanoparticles wt.% content in Nanocomposite

Figure (3) demonstrated the effect of addition different weight fraction of nanoparticles w.t% (GNP and HAp) on compression strength. The compression strength increased with addition PAN fibers and with increased of weight fraction of HAp% also increased with GNP% up to 0.8% and after that the compression strength slightly decreased. Moreover, the increment in compression strength at addition HAp% was higher than at addition of GNP% due to the nature of nanoparticles and the Lubricant properties of GNP which made it more susceptible to compression than HAp nanoparticles
The figure (4) illustrate the hardness increased with addition PAN fibers and increased weight fraction of GNP% and HAp% due to strong interfacial bond between the matrix and the reinforcement allowing efficient load transfer mechanism. Also nanoparticles have good mechanical properties that can influence the properties of the matrix positively thus lead to transfer the load from matrix to nanoparticles. [19,20]

The hardness at addition HAp was higher than at addition GNP due to natural HAP is a ceramic brittle material.
Figure (5) show the bacterial inhabitation area of pure ethanol (a) and neat UHMWPE, composite material (UHMWPE+5%PAN fiber) (b) and nanocomposite material reinforced with different weight fraction wt.% of GNP and HAp (c and d) respectively. which illustrated there was not inhibition of bacterial for all solid compressed samples because the agar needed to media to diffuse within it but the natural of UHMWPE had low absorbedly thus at compress sample the agar cannot diffusion within it. And also there was not inhibition area for ethanol alone. Table 1 exhibited the anti-bacterial activity of neat UHMWPE, composite material (UHMWPE+5%PAN fiber) and nanocomposite material reinforced with different weight fraction wt.% of GNP and HAp. The neat polymer had good antibacterial activity and the composite material (UHMWPE+5%PAN fiber) cause to increase the diameter of inhabitation of E-coli Bacteria due to PAN fiber had antibacterial properties[21]. while, diameter of inhibition decreased at addition 0.3% of nano particles (GNP and HAp) but with increase the weight fraction of Nanoparticles, the diameter of inhibition increased and become more stable especially at 0.8% for GNP% and for HAp% start increased and stable after over 0.3 w.t HAp [22]. Moreover, the antibacterial activity of HAp is more than GNP which attributed to the particles size of HAp is about 20 nm moreover, the HAP nanoparticles activate the bacterial grovel and spores so that the efficiency of bactericidal became higher [22].
Figure (5) illustrated the inhibition area neat polymer and composite material for E. coli and Staphylococcus colony.

Table (1) Inhibition Zone for Pure Ethanol and Hybrid Nanocomposites.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Inhibition zone diameter (mm)</th>
<th>E-coli</th>
<th>Staphylococcus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure Ethanol</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Neat UHMWPE</td>
<td>25</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>UHMWPE+5%PAN</td>
<td>26</td>
<td>30</td>
<td>30</td>
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<td>15</td>
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<td>UHMWPE+5%PAN+0.8%GNP</td>
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<td>UHMWPE+5%PAN+1.3%GNP</td>
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<tr>
<td>UHMWPE+5%PAN+0.3%HAp</td>
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<td>UHMWPE+5%PAN+0.8%HAp</td>
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<td>UHMWPE+5%PAN+1.3%HAp</td>
<td>24</td>
<td>22</td>
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</tr>
</tbody>
</table>

**Conclusion**

The hybrid nanocomposite was enhanced the mechanical properties (flexural strength, flexural modulus, compression strength and hardness) to support the weight of patient and improved the performance of knee joint replacement by develop the properties of cartridge and enhance the anti-bacterial properties to avoid the infection after implantation process that cusses the failed joint replacement.

**Ethical Clearance:** I am studied ten case of patient that proceed surgical operation to replace knee joint in (Medical City Hospital) for knowing the important notes, the defects of joint and the service life of synthetic joint before replacement.

**Acknowledgement:** I would like to thank Medical City Hospital and I would like to express my gratitude to Dr. Montadher Najim /Ph.D in Orthopaedic Surgery that help me for obtain information about knee joint replacement

No **Conflict Interesting**

No **Source Funding** (Self).

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The Relationship of Age, Working Periode, and Work Attitude with Complaints of Carpal Tunnel Syndrome on Workers in the Sumenep Batik Industry Indonesia

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Abstract

Making batik pattern is a job involving hand pinning the canting spout (a small metal cup with a wooden handle) and bending. Unnatural working position for a long time can cause a variety of health problems to workers, one of which is the hands. This can cause Carpal Tunnel Syndrome (CTS) which is closely related to repeated and excessive use of hands. The purpose of this study was to analyze the relationship of age, working period and work attitude with complaints of carpal tunnel syndrome on workers in the Sumenep Batik Industry.

This study is an analytical research using observational methods and through a cross sectional approach. The population in the study were all worker in batik who worked using canting. The sample was 32 workers out of 47 workers in the population. Data was collected using interview, questionnaires, panel tests, and work attitude measurements using the RULA observation sheet.

The results showed that 68.8% of respondents experienced complaints of Carpal Tunnel Syndrome. The majority of CTS complaints were from respondents aged 36-45 years, working for more than 10 years, and having high risk work attitude.

The conclusion is that there is a relationship between age, working period and work attitude with CTS complaints. Industrial owners are expected to prepare the same number of melted-wax pan and the workers, so that the workers will have more ergonomic work attitudes. Workers are suggested to take short breaks, do CTS exercises, to immediately visit to the nearest health center if they experience CTS complaints.

Keywords: carpal tunnel syndrome, working periode, work attitude, worker in batik

Introduction

Carpal Tunnel Syndrome (CTS) is a medical disorder that occurs due to suppression of the median nerve in the carpal tunnel with the main symptoms of tingling and pain in the fingers and hands which are innervated by the median nerve. The other symptoms are numbness, muscle weakness, stiffness and the possibility of muscle atrophy\(^1\). The prevalence rate of CTS is 276 in 100,000 per year. CTS is more common in women than men and often occurs bilaterally with an increased incidence between the ages of 40-60\(^2\).

Based on the American Academy of Orthopedic Surgeons report (2009), the incidence of carpal tunnel syndrome in the United States was estimated to be 1-3 cases per 1,000 subjects per year. However, the prevalence of CTS in Indonesia is still unknown due to very few diagnoses of occupational complaints were reported\(^3\).

Manufacturing industry is a group of types of business that process materials into semi-finished goods or finished goods which have greater added value\(^4\). One of the manufacturing industries in Indonesia is the batik industry. Worker in batik are informal sector workers who traditionally process various types of
cloth into batik. They draw and design the batik, make the batik pattern using the melted wax, color the batik using dyes and dry it using traditional methods\(^5\). One of the batik industries in Indonesia, especially East Java, is the Sumenep Batik Industry. Here, worker in batik work with the position of the hands pinning the canting pen and bending in the process of making batik. This position makes the wrist bear heavy load.

Age is one of the factors that can increase the number of CTS complaints. According to Bridger (2003), the older a person is, the higher the risk of the person experiencing a decrease in bone elasticity, so that it becomes a trigger for symptoms that include MSDs, one of them is CTS\(^6\). In addition, working period and work attitudes are also among the factors that can trigger CTS complaints.

According to Budiono (2003), the working period is divided into 3 categories, namely new (less than 6 years), moderate (6-10 years), and long (more than 10 years)\(^7\). The longer the working period, there will be more continuously repeated movements of the finger for a long time, causing stress on the tissue around the carpal tunnel\(^8\). In addition, worker in batik often make sideways movements to obtain batik candles during the waxing process due to the stove position which is quite far from the workers. If this work attitude is carried out continuously, workers will easily experience the risk of Carpal Tunnel Syndrome (CTS) complaints\(^6\).

The description above shows that worker in batik have the potential danger of complaints of Carpal Tunnel Syndrome (CTS). Thus, this research was aimed to analyze the relationship of age, working period and work attitudes with complaints of Carpal Tunnel Syndrome (CTS) in workers in the Sumenep Batik Industry.

The purpose of this study was to analyze the relationship of age, working period, work attitude and Carpal Tunnel Syndrome (CTS) complaints on workers in the Sumenep Batik Industry.

**Material and Method**

This research is an observational study with a cross sectional approach. The population in this study were 47 worker in batik who worked using canting pen in the Sumenep Batik Industry. The sample in this study were 32 people.

A sample of 32 workers was taken by the Simple Random Sampling technique, which is taking samples from the population randomly drawn without regard to the strata in the population\(^9\). The sample size was determined using the following formula:

\[
\begin{align*}
    n &= \frac{(Z_{1-\alpha})^2 \times p \times q \times N}{d^2(N-1) + (Z_{1-\alpha})^2 \times p \times q} \\
    \text{Statement:} \\
    (Z_{1-\alpha})^2 &= \text{The value of Z on the normal curve to } \alpha = 0.05 \text{ confidence coefficient } 95\% \text{ by } 1.96 \\
    p &= \text{The proportion of the value of 0.5} \\
    q &= 1-p = 1-0.5 = 0.5 \\
    d &= \text{Degree of precision} \\
    N &= \text{The total number of the population } = 47 \text{ workers} \\
    n &= \text{The number of samples} \\
    n &= \frac{(1.96)^2 \times 0.5 \times 0.5 \times 47}{0.1^2(47 - 1) + (1.96)^2 \times 0.5 \times 0.5} \\
    \text{or } n &= 31.77 = 32 \text{ workers}
\end{align*}
\]

The variablers of this research were age, working period, work attitude and Carpal Tunnel Syndrome (CTS) complaints. The instruments used in collecting the data were interview, questionnaires, panel tests, and the RULA observation sheet.

To analyze the data, Kendall’s Tau-b test was employed. A significance value was obtained from the test. Then, the significance value is compared with the \(\alpha\) value, which is equal to 5% or 0.05. If the significance value obtained is less than the value of \(\alpha\), the two tested variables have a correlation. If the significance value is more than the value of \(\alpha\), then the two tested variables have no correlation.

To find out the strong of the relationship between
independent variables and the dependent variable, the contingency coefficient (CC) of the symmetric measure table was used. According to Sugiyono (2010) the category of CC value is divided into:

**Tabel 1. Contingency Coefficient**

<table>
<thead>
<tr>
<th>The Value of Contingency</th>
<th>Level of The Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00 – 0.199</td>
<td>Very weak</td>
</tr>
<tr>
<td>0.20 – 0.399</td>
<td>Weak</td>
</tr>
<tr>
<td>0.40 – 0.599</td>
<td>Moderate</td>
</tr>
<tr>
<td>0.60 – 0.799</td>
<td>Strong</td>
</tr>
<tr>
<td>0.80 – 1.00</td>
<td>Very Strong</td>
</tr>
</tbody>
</table>

**Findings**

There were 32 respondents in this study. They were workers in the Sumenep Batik Industry. The descriptions of the respondents are as follows:

Based on the research, the distribution of the respondents is as shown below:

**Tabel 2. Distribution of Respondents Based on Age, Working Period, and Work Attitude in The Sumenep Batik Industry**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>17 – 25</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>26 – 35</td>
<td>6</td>
<td>18.8%</td>
</tr>
<tr>
<td></td>
<td>36 – 45</td>
<td>14</td>
<td>43.8%</td>
</tr>
<tr>
<td></td>
<td>46 – 55</td>
<td>7</td>
<td>21.9%</td>
</tr>
<tr>
<td></td>
<td>56 – 65</td>
<td>5</td>
<td>15.6%</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>32</td>
<td>100%</td>
</tr>
</tbody>
</table>

| Working Period | < 6 years | 9 | 28.1% |
|               | 6–10 years| 9 | 28.1% |
|               | > 10 years| 14| 43.8% |
| Total          |          | 32| 100%  |

| Work Attitude  | Low      | 0 | 0%  |
|                | Moderate | 7 | 21.9%|
|                | High     | 19| 59.4%|
|                | Very High| 6 | 18.8%|
| Total          |          | 32| 100% |

Table 2 shows that majority of respondents (43.8%) are 36 – 45 years old and have been working in the industry for more than 10 years. This table also shows that most of the workers (59.4%) have CTS high risk work attitude.

Complaints of Carpal Tunnel Syndrome (CTS) were obtained using questionnaires and panel tests. Table 3 shows that respondents who reported CTS complaints were 22 workers (68.8%), while those who reported no CTS complaints were 10 workers (31.3%).

**Tabel 3. Distribution of Complaints of CTS**

<table>
<thead>
<tr>
<th>No</th>
<th>Complaints of CTS</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yes</td>
<td>22</td>
<td>68.8%</td>
</tr>
<tr>
<td>2</td>
<td>No</td>
<td>10</td>
<td>31.3%</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>32</td>
<td>100%</td>
</tr>
</tbody>
</table>

Complaints of Carpal Tunnel Syndrome (CTS) were obtained using questionnaires and panel tests. Table 3 shows that respondents who reported CTS complaints were 22 workers (68.8%), while those who reported no CTS complaints were 10 workers (31.3%).

Bivariate analysis produces data relating to the relationship between independent variables consisting of age, working period and work attitudes, which are associated with the dependent variable, namely Carpal Tunnel Syndrome complaints. The statistical test used was the Kendall’s Tau-b.

Statistical analysis using the Kendall’s Tau-b test obtained a significance value of 0.005 at the level of error (α) = 5% for the correlation of age variable and CTS complaints. It means that there is a correlation between age and CTS complaints. Level of relationship closeness is shown by the value of the contingency coefficient, which is 0.497. This means that the correlation between age and CTS complaints is a moderate and in a positive direction.

The correlation of working period and CTS complaints has a significance values of 0.00 at the error level (α) = 5% and the contingency coefficient (CC) of 0.614. This means that the correlation between work period and CTS complaint is strong and positive.

The correlation of working attitude and CTS complaints has a significance values of 0.00 at the error level (α) = 5% and the contingency coefficient (CC) of 0.542. This means that the correlation between work attitude and CTS complaint is moderate and positive.
Discussion

Carpal Tunnel Syndrome (CTS) is a disorder caused by excessive use of hands for a long time period\(^{(10)}\). Age factors, working period, and work attitudes are some of the factors that can cause CTS\(^{(6)}\).

According to the Ministry of Health (2009), age categories are divided into five categories, namely\(^{(11)}\):

- **a) Late adolescence**: 17-25 years
- **b) Early adulthood**: 26-35 years
- **c) Late adulthood**: 36-45 years
- **d) The early elderly period**: 46-55 years
- **e) The final elderly period**: 56-65 years

In this study, the majority of respondents who experienced CTS were in the age category of 36-45 years or 34.4%.

Statistical test found that there was a moderate and positive correlation of age and CTS complaints. This means that the more you age, the more you will experience CTS complaints. Making batik requires special creativity and expertise from workers. Therefore, at the end of their late adulthood to elderly age, workers tend to be more skilled and quite skilled in making batik as they have accustomed with the works for years. This means that respondents are at more risk of experiencing CTS complaints at this age.

This findings support Tarwaka (2015) who stated that the first muscle complaints is more likely experienced by someone at the age of 30 years. Starting from this age, the level of complaints will always increase along with the age. This happens because muscle strength and endurance begin to decrease at middle age, so the risk of muscle complaints also increases from this age\(^{(12)}\). This findings also confirm and support the findings of Ibrahim et al (2012) which stated that CTS often occurs between the age of 40-60 years old\(^{(2)}\). In addition, this findings is also in line with what Dhaniswara (2017) found that there is a significant relationship between age and incidence of CTS in worker in batik and those who experience a greater incidence of CTS are aged 41-60 years\(^{(13)}\).

The correlation statistical result between working period and complaints of CTS shows a positive relationship. This means that the longer the working period, the higher the risk of experiencing CTS complaints. According to Budiono (2003), the working period is divided into 3 categories, namely new (<6 years), moderate (6-10 years), and long (> 10 years)\(^{(7)}\).

The correlation between working period and CTS complaints is a positive and strong. The majority of respondents who reported CTS complaints are the workers with more than 10 years period of working (40.6%). This is due to the repeated and excessive hand and fingers movements for a long time period, which then causes the tissue around the carpal tunnel\(^{(8)}\).

This findings is in line with the findings of Dhaniswara (2017), which stated that there is correlation between working period and CTS complaints\(^{(13)}\). This finding is also supported by Angelia (2014) who found that the correlation between working period and CTS complaints is strong. There were 74.4% of his respondents who worked for more than 10 years reported most of complaints of CTS compared to the other workers who had less working period\(^{(14)}\).

Another factor that can also cause CTS is work attitude. Measurements of work attitude can be done by several methods. One of them is using the RULA (Rapid Upper Limb Assessment) method. RULA is a research method for investigating disorders of the upper limb. The method was designed by Lynn Mc Atamney and Nigel Corlett (1993) who provided a calculation of musculoskeletal load levels in a job that can cause CTS complaints, a risk to body parts from the abdomen to the neck or upper limbs including the wrist\(^{(15)}\). This method is very suitable to be applied to a static work such as batik. According to Tarwaka (2015), the results of the RULA assessment are categorized into 4, namely low, moderate, high, and very high\(^{(12)}\).

The statistical test result shows that there is a moderate and positive correlation between work attitudes and CTS complaints. This means that the more high-risk work attitudes, the higher the risk of experiencing CTS. The work attitudes of most of respondents (46.9%) were in the high-risk category and they reported most of CTS complaints.

This findings confirms findings of Agustin (2012) which stated that there was a moderate correlation between work attitude and CTS complaints on the worker in batik\(^{(16)}\).
Conclusion

It can be concluded that of the 32 respondents who work in the Sumenep Batik Industry:

1. The majority of them are aged 36-45 years (14 workers), have been working for more than 10 years (14 workers), and have high risk work attitudes (19 workers)
2. It also can be concluded that there is a positive and moderate correlation between age and CTS complaints
3. Strong correlation between working period and CTS complaints.
4. Moderate correlation between work attitude and CTS complaints on the worker in batik

Recommendation

1. To apply ergonomic work attitude for the workers’ wrist.
2. We suggest the industrial owners to prepare melted-wax pan the same number of workers so that workers do not need to make un-ergonomic (sideways or twisting) movement to take the wax.
3. It is recommended for the workers to take regular break every 15-20 minutes by bending and straightening the wrist. Agustin (2012) suggested batik workers to do exercises by clenching their fists, bending the wrists downward and upward and holding for 30 seconds\(^1\).
4. It is better for workers who experience CTS to immediately report their complaints to the nearest health care or doctor, so that their complaints are immediately followed up with medication and prevention.

Conflict of Interest: All authors have no conflicts of interest to declare.

Source of Funding: This is an article “The Relationship Between Rest and Work Attitudes with Carpal Tunnel Syndrome (Case of Batik Craftsmen in Batik Industry Pekandangan Sumenep)” of Occupational Health and Safety Department that was supported by Faculty of Public Health, Airlangga University.

Ethical Clearance: The study was approved by the institutional Ethical Board of the Public Health, Airlangga University.

References


Comparative Analysis Study of Different Positions by Hands and Feet on Functional Mobility Assessed by Five Times Sit to Stand Test on Post-Stroke Patients

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Abstract

Background: Five times sit to stand test (FTSTS) has been recognized for its validity and reliability in assessing muscle strength of the lower extremities functionally, evaluating functional mobility, balance and risk of falling in post-stroke individuals. However, there is no standard protocol for FTSTS implementation.

Objectives: To analyze the differences in functional mobility assessed with FTSTS results in various post-stroke post-hand posture and posture changes.

Method: There were 11 patients ischemic stroke patients enrolled in the study. The age ranked from 40-60 years old. This study used an observational and cross-sectional study. The characteristic and FTSTS data was recorded in the Five times sit to stand test form that designed for this study. The data will then be analyzed using paired t-test in groups with SSPS 16.0 program.

Results: The paired t-test results show that there are significant differences between the FTSTS results in both hand positions, either on the spontaneous feet position or asymmetric feet position (p = 0.038 and 0.020). There are no significant differences in FTSTS results with changes in spontaneous or asymmetric feet position (p = 0.779 and 0.278).

Conclusion: The augmented hand position gives the best result to shift the center of the body mass on the post-stroke patient with post-hand changes. Then, there was no difference in functional mobility assessed by FTSTS in feet position changes of the post-stroke patient.

Keywords: FTSTS, Stroke, Hand position, Feet position

Introduction

Clinically the most common symptom in stroke patients is the presence of hemiparesis, which results in the loss of normal postural reflex mechanisms for balance and body rotation for functional movement of the extremities 1,2. The previous study in 161 elderly stroke patients, showed 37% fell during a change in position such as getting up from a chair. As a consequence, improving the symmetry of body load distribution and balance becomes the goal of rehabilitation in stroke 3–6. Related to the purpose of stroke rehabilitation, the clinician requires a valid and reliable measurement to know and evaluate the motor status and functional mobility of the patient7. The ability to get up from the chair is a prerequisite for independent ambulation and more functional daily life activities such as meals,
attending an activity and while on public transport. This transition movement becomes more difficult in individuals with post-stroke hemiparesis.

FTSTS has been recognized for its validity and reliability in assessing functional lower extremity muscle strength, evaluating functional mobility, balance and risk of falling in healthy elderly individuals, stroke and Parkinson’s. Although it has been commonly used as an output parameter, there is no standard of FTSTS implementation protocol. The position of the hand when the subject performs the FTSTS is inconsistent. In most studies, subjects were asked to fold or cross their arms in the chest. While, in another study, subjects were asked to place their hands on their laps or simply stand without using hands during FTSTS. In fact, some studies do not mention the position of the hand at all.

In this study, our aim is to compare the results of FTSTS with the various hand and feet position after stroke when performing the FTSTS. We also add a condition where the augmented hand positions with asymmetry feet positions have not been studied together before. It is hoped that this research could provide information to physicians and physiotherapists in improving services in post-stroke patients at Dr. Soetomo General Hospital Surabaya, Indonesia.

Method

This research was an observational analytic with cross-sectional study approach that performed in outpatient unit of Medical Rehabilitation Installation at Dr. Soetomo Teaching Hospital Surabaya, Indonesia. The characteristic and FTSTS data were taken from August to November 2015. The subjects were first chronic ischemic stroke-patients, with hemiparesis aged 40-60 years, understanding the command, able to stand independently from the chair without the help of hands, stable general medical conditions, and willing participant in this research by the signing of informed consent.

The subjects were consecutive sampling and the number of samples obtained by 11 patients. Ethical appeals were submitted to the Ethics Commission for basic science / clinical clearance research at Dr. Soetomo General Hospital Surabaya, Indonesia. The data was recorded in the Five times sit to stand test form that designed for this study. The data will then be analyzed using paired t-test in groups with SSPS 16.0 (SPSS, Inc., Chicago, IL) program.

Results

Functional mobility capability (FTSTS results) of post stroke

All study subjects performed by FTSTS according to the study protocol in the following order: condition 1 was the position of the spontaneous feet and arms across the chest; condition 2 was the position of the spontaneous feet and the position of the hand augmented; condition 3 was the position of the feet of asymmetry and the position of the hand across the chest; and condition 4 was the position of the feet asymmetry and hand positions augmented. The average FTSTS results for 4 conditions ranged from 9.2 ± 2.8 to 10.3 ± 2.9 seconds (4.8 to 16.1 seconds). However, in condition 2 was the spontaneous feet position and the augmented hand gave the FTSTS the shortest result with 9.2 ± 2.8 seconds.

FTSTS Results at Different Hand Positions

Based on paired t-test results, there was a significant difference between FTSTS results in both hand positions, either on the spontaneous feet position or asymmetric feet position (p = 0.038 and 0.020) (Table 3).

FTSTS Results at Different Feet Positions

In this study, there was no significant difference in FTSTS results with changes in spontaneous or asymmetric feet position. The paired t-test of the change of the position of the feet against the FTSTS results is shown in Table 4.

Table 1. Subject Characteristics of Ordinal Data Research

<table>
<thead>
<tr>
<th>Variables</th>
<th>Amount (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>7 (6.36)</td>
</tr>
<tr>
<td>Female</td>
<td>4 (36.4)</td>
</tr>
<tr>
<td>Hemiparesis Sides</td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>5 (45.5)</td>
</tr>
<tr>
<td>Left</td>
<td>6 (54.5)</td>
</tr>
<tr>
<td>Atrophy AGB parese</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2 (18.2)</td>
</tr>
<tr>
<td>No</td>
<td>9 (81.8)</td>
</tr>
</tbody>
</table>
Table 1. Subject Characteristics of Ordinal Data Research

| Ankle spasticity | 0 | 5 (45.5) |
| 1 | 4 (36.4) |
| +1 | 2 (18.2) |

A walker tools

| Cane | 1 (9.1) |
| None | 10 (90.9) |

Table 2. Subject Characteristic of Age, Weight, Height, Mass Index, Length of lower feet, and Post-stroke Duration

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean of ±SD Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y/o)</td>
<td>51.0 ± 5.5 [40 – 58]</td>
</tr>
<tr>
<td>Body weight (Kg)</td>
<td>64 ± 17.01 [39 – 91]</td>
</tr>
<tr>
<td>Body height (cm)</td>
<td>157.54 ± 7.10 [148 – 170]</td>
</tr>
<tr>
<td>Body Mass Index (Kg/m2)</td>
<td>26.04 ± 6.55 [16 – 39]</td>
</tr>
<tr>
<td>Length of the lower feet (cm)</td>
<td>37.63 ± 3.14 [33-42]</td>
</tr>
<tr>
<td>Duration of post-stroke (week)</td>
<td>74.50 ± 24.0 [41 – 104]</td>
</tr>
</tbody>
</table>

Table 3. The paired t-test results in hand position changes to the FTSTS results

<table>
<thead>
<tr>
<th>No. Feet position</th>
<th>Hand position</th>
<th>FTSTS results Comparing</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Spontaneous</td>
<td>Crossed in the chest</td>
<td>10.1 ± 2.8</td>
<td></td>
</tr>
<tr>
<td>2 Spontaneous</td>
<td>Augmented</td>
<td>9.2 ± 2.8</td>
<td>0.038*</td>
</tr>
<tr>
<td>3 Asymmetry</td>
<td>Crossed in the chest</td>
<td>10.3 ± 2.9</td>
<td></td>
</tr>
<tr>
<td>4 Asymmetry</td>
<td>Augmented</td>
<td>9.5 ± 2.9</td>
<td>0.020*</td>
</tr>
</tbody>
</table>

Note: * = significant, \( p < 0.05 \)

FTSTS: Five times sit to stand test

Table 4. The changes feet position result of the t-test’s pair to the FTSTS

<table>
<thead>
<tr>
<th>No.</th>
<th>Feet position</th>
<th>Hand position</th>
<th>FTSTS results</th>
<th>Comparing</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Spontaneous</td>
<td>Crossed in the chest</td>
<td>10.1 ± 2.8</td>
<td>Condition 1 - Condition 2</td>
<td>0.779</td>
</tr>
<tr>
<td>3</td>
<td>Spontaneous</td>
<td>Augmented</td>
<td>9.2 ± 2.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Asymmetry</td>
<td>Crossed in the chest</td>
<td>10.3 ± 2.9</td>
<td>Condition 3 - Condition 4</td>
<td>0.278</td>
</tr>
<tr>
<td>4</td>
<td>Asymmetry</td>
<td>Augmented</td>
<td>9.5 ± 2.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FTSTS: Five times sit to stand test

Discussion

Based on paired t-test results, there were significant differences in FTSTS results with hand position changes in post-stroke patients. Between the two hand positions assessed in this study, the augmented hand positions gave shorter results than the crossed position in the chest (Table 3). This result was in accordance with research by Kwong and his friends that augmented hand positions help shift the centre of the body mass forward more efficiently. Previous research on sitting
to standing movements is often performed by limiting the use of the hands. The centre of the body mass moves forward at the end of the sitting to stand while the subject is pointing with their hands. Hand restrictions cause different patterns of angular changes in ankle joints, a much higher standard deviation than in the free arm that obtained. The position of the augmented arm is the position of both hands interlocked with shoulder flexion 90° and full extension elbow. This augmented hand position is often used in functional mobility exercises with the Bobath method.

The mean of time to complete the FTSTS in this study was ranged from 9.2 ± 2.8 seconds to 10.3 ± 2.9 seconds. These results were shorter than those of Mong study who received mean FTSTS in post-stroke patients was 17.1 ± 7.5 seconds in the mean post-stroke subjects aged 60.0 ± 4.8 years. Similar results were obtained in the previous study that the mean of FTSTS was 15.2 ± 17.1 seconds, with age of study subjects ranging from 60 ± 5.6 years. Similarly, research by Ng in post-stroke patients with mean age between 60.0 ± 5.56 (50-70 years) obtained FTSTS results between 15.81 to 18.20 seconds. FTSTS results in this study could be due to post-stroke patients that included the younger age of 51.9 ± 7.3 years (40 - 58 years).

Previous study did not take into account the height of the chair and using a standard chair by 43 cm. However, in this study using an especially adjustable seat height between 41 - 47 cm with seat height was 100% of the length of the lower limbs of the study subjects. According to Ng, the lower the seat, the longer it takes to complete the FTSTS. The lower seats the lower the centre of body mass and increase the degree of togok flexion and the angle of flexibility movement of togok, hip joints, knee joints and ankles while sitting to standing. As it moves from sitting to standing, it takes a greater increase of greater moments on the hip joint and knee joint.

It is obtained that sitting down to standing was required shorter time. The posterior positioning of the feet also gives the hip joint flexion and flexion speed of the smaller hip joint. Another study examined the effect of early posterior feet placement. The posterior position of the feet causes the maximum mean moment of extension of the lower hip joint. Early feet placement also affects the travel distance of the centre of gravity (CoG). Upon awakening, the movement of the centre of gravity of the body forward during standing was significantly longer on normal feet placement when compared to posterior feet placement, with reference to 10 cm behind the normal position.

In this study, there was no significant differences in FTSTS results with 2-feet positions, spontaneous and asymmetric feet position with parese feet that placed behind. Similar Lecours et al. study, on subjects with hemiparesis that has 2 possibilities when the asymmetry of the feet was positioned on the task of sitting to stand and standing up to sit. First, the subjects do a weight bearing on the position of the feet that was in the posterior as well as healthy people. Second, naturally subjects still place weight bearing on healthy feet, although “forced” weight bearing on parent feets. In this study, the second possibility has occurred.

According to previous study, it suggested the presence of weight-bearing asymmetry in patients with hemiparesis. These results are supported by evidence of togok asymmetry towards the healthy side. In this study, the patient performs a sitting motion to stand by uniforiming the starting position by starting leaning on the back of the chair, but not instructing to return again on leaning back in the seat during sitting motion, during the FTSTS. According to the researchers, there could be asymmetry togok toward the healthy side that helps stability when subjects perform FTSTS and give short results, without affected feet position.

**Conclusion**

There was a difference in functional mobility assessed by FTSTS on the post-stroke patient with post-hand changes where the augmented hand position gives the best result to shift the center of the body mass. Then, there was no difference in functional mobility assessed by FTSTS in feet position changes of the post-stroke patient.

**Ethical Clearance:** This research involves participants in the process using a questionnaire that was accordant with the ethical research principle based on the regulation of research ethic regulation. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, non-maleficence, and justice.

**Conflict of Interest:** The authors have not found any conflict of interest related to this research so far.
Source of Funding: All of the cost and fees related with this research are paid by the authors only with no sponsorship nor external funds.

References


Relationship between Fatigue and Musculoskeletal Complaints on Pedicab drivers in the Pedicab Association Solo Balapan Station

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2Faculty of Health Science, Muhammadiyah University, Surakarta, Indonesia

Abstract

Musculoskeletal complaints can occur when muscles or skeletons receive a load or work is done repeatedly and the work is done for a long time. Pedaling a pedicab every day for a long time can cause fatigue. The fatigue felt by the pedicab drivers will have an impact on the pain in the muscles both in the legs, back, hands, shoulders, and neck. The aim of the study was to determine the relationship between the level of work fatigue and musculoskeletal complaints on pedicab drivers in the pedicab association Solo Balapan Station. This type of research is quantitative research. The research method used observational research. The study design was cross-sectional. The population in this study were 35 pedicab drivers, a sample of 33 people. Instrument research uses a questionnaire. Data analysis used the test Fisher exact. The results showed that there was a relationship between the level of work fatigue and musculoskeletal complaints (p=0.001). There is no relationship between age of workers and musculoskeletal complaints (p=1). There is no relationship between work periods drivers and musculoskeletal complaints (p=1). There is no relationship between the length of work/day with musculoskeletal complaints (p=0.273). There is no relationship between the average distance traveled with musculoskeletal complaints (p = 0.273)

Keywords: Fatigue, Musculoskeletal, Pedicab drivers

Introduction

The human body is designed to be able to carry out daily work activities. The existence of a muscle mass that weighs more than half the weight of the body makes it possible to move the body and do work. Every job is a burden for the person concerned. The burden is in the form of physical workload and mental workload. In practice the workload encountered is a combination of physical burden and mental burden1.

Fatigue is a condition characterized by feeling tired and decreasing alertness and influencing work productivity. According to Grandjean (1993) work fatigue is a symptom characterized by feelings of fatigue and decreased alertness2.

Exhaustion of work can have an impact on decreased attention, slowing and barriers to perception, slow and difficult thinking, decreased motivation to work, decreased alertness, decreased concentration and accuracy, low work performance, low work quality, and decreased reaction speed. These things will cause a lot of mistakes so that workers experience musculoskeletal complaints, work stress, work-related diseases, workplace accidents, and ultimately productivity decreases3.

According to Setyawati (2010), work fatigue occurs due to the accumulation of lactic acid. At work the body needs energy. The energy is obtained from the results of glycogen breakdown. In addition to energy, lactic acid is one result of the breakdown of glycogen. When the muscles contract, the lactic acid buildup will occur. This lactic acid inhibits muscle action and causes fatigue4.

Work exhaustion is a condition of weakening activities, motivate, and physical fatigue to do work. According to Cameron (1973) in Setyawati (2010), work fatigue involves a decrease in physical performance, a feeling of fatigue, a decrease in motivation, and a decrease in work productivity4. Work exhaustion cannot be defined but can be felt so that the determination of
work fatigue can be known subjectively based on the feelings experienced by the workforce. According to Suma’mur (2009), that work fatigue does not only occur at the end of work time but can also occur before work.

Complaints of the musculoskeletal system are complaints to parts of the skeletal muscles that are felt by someone starting from very mild complaints to very sick. If the muscle receives a static load repeatedly over a long period of time it will cause complaints in the form of damage to joints, ligaments, and tendons.

Work activities carried out by pedicab drivers include pedaling and lifting weights. During pedaling activities, the posture is sitting upright or bent, right and left foot pedaling alternately, with a pattern of movement of both feet pedaling, and intense limbs moving the hips, thighs, calves, soles of the feet. When the pedicab lifts the weight, the posture is in the position of the back straight or bent, hands raised, feet become the foundation of the body with a pattern of movement of the hand holding the load and lifting it, and intense limbs move the hands, back, and legs. As a result of the activity of pedaling a pedicab, pedicab drivers can be at risk of developing musculoskeletal complaints. Based on the background, the researchers wanted to examine further the relationship between the level of work fatigue and musculoskeletal complaints on pedicab drivers in the pedicab association Solo Balapan Station. The purpose of this study was to determine the relationship between the level of work fatigue with musculoskeletal complaints on pedicab drivers in the pedicab association Solo Balapan Station.

Material and Method

This study is an observational study. The design in this study uses cross-sectional. The population in this study are all 33 pedicab drivers who are members of the association of Solo Balapan Station rickshaw. The sampling technique used in this study was using total bivariate analysis using the Fisher exact test.

Findings

3.1 Characteristics of Respondents

<table>
<thead>
<tr>
<th>Variable</th>
<th>Amount</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risky</td>
<td>32</td>
<td>97.0</td>
</tr>
<tr>
<td>No risky</td>
<td>1</td>
<td>3.0</td>
</tr>
<tr>
<td>Fatigue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>19</td>
<td>57.6</td>
</tr>
<tr>
<td>Negative</td>
<td>14</td>
<td>42.4</td>
</tr>
<tr>
<td>Musculoskeletal complaints</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>21</td>
<td>63.6</td>
</tr>
<tr>
<td>Negative</td>
<td>12</td>
<td>36.4</td>
</tr>
<tr>
<td>Length of work</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Old</td>
<td>30</td>
<td>90.9</td>
</tr>
<tr>
<td>New</td>
<td>3</td>
<td>9.1</td>
</tr>
<tr>
<td>hours per day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risky</td>
<td>19</td>
<td>57.6</td>
</tr>
<tr>
<td>No risky</td>
<td>14</td>
<td>42.4</td>
</tr>
<tr>
<td>mileage / day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long</td>
<td>22</td>
<td>66.7</td>
</tr>
<tr>
<td>Short</td>
<td>11</td>
<td>33.3</td>
</tr>
</tbody>
</table>

Based on Table 1 unknown 32 respondents (97%) > 35 years old, while 1 respondent (3%) does not include the age at musculoskeletal risk. It is known that 19 respondents (57.6%) experienced mild fatigue. A total of 14 respondents did not experience fatigue (14%). Many respondents complained of musculoskeletal pain as many as 21 people (63.6%), 12 respondents did not complain musculoskeletal (36.4%). The length of work known to 30 respondents (90.9%) have long been pedicab drivers. 3 respondents worked <7 years (9.1%). Many respondents worked 8 hours per day as many as 19 people (57.6%) while 14 people worked <8 hours per day (42.4%). A total of 22 respondents (66.7%) pedaled pedicabs in long distances, while 11 respondents (33.3%) pedaled pedicabs in short distances.
Table 2. Relationship between fatigue and complaints musculoskeletal on pedicab drivers at Solo Balapan Station

<table>
<thead>
<tr>
<th>Fatigue</th>
<th>Musculoskeletal complaints</th>
<th>N</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>17</td>
<td>2</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>89.5%</td>
<td>10.5%</td>
<td>100%</td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>28.6%</td>
<td>71.4%</td>
<td>100%</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>12</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>63.6%</td>
<td>36.4%</td>
<td>100%</td>
</tr>
</tbody>
</table>

The results of testing the statistics are Fisher Exact known to be $p = 0.001$ ($p < 0.05$) with 95% CI = 3.280-137,667. These results indicate fatigue associated with musculoskeletal complaints in pedicab drivers at Balapan Station.

Based on the results of the study it was found that there was a relationship between fatigue and musculoskeletal complaints on pedicab drivers at Balapan Station. The results of this study explain fatigue as one of the factors causing musculoskeletal disorders. Fatigue is a condition where the body feels tired and not energized at any given time. This condition is actually a warning or sign from the body so that the body can be rested for a moment. Important rest is done as an effort so that the body can avoid more serious damage due to fatigue\(^6\). Musculoskeletal disorders can develop into disability and loss of ability and working time\(^7\).

Symptoms Muscle fatigue can be seen in symptoms that appear from the outside. There is a general theory of chemistry explaining that fatigue is a result of reduced energy reserves and increased metabolic waste as a cause of loss of muscle efficiency. While changes in electric current in the muscles and nerves are secondary causes. Whereas the central nervous theory explains that chemical changes are only supporting processes. Chemical changes that occur result in the delivery of nerve stimulation through sensory nerves to the brain that is recognized as muscle fatigue. This afferent stimulation inhibits the brain centers in controlling movement so that the potential frequency of activity in nerve cells is reduced. The reduced frequency will reduce the strength and speed of muscle contraction and movement on the orders of will to be slow. This the slower the movement of a person will show the more tired the condition of one’s muscles. With conditions that are tired of the respondent, the greater the experience of musculoskeletal complaints.

The results of this study indicate that failure is associated with musculoskeletal complaints. The fatigue of these respondents can also be caused by the existence of a long working period of more than 8 hours a day. This condition is very possible for workers to experience musculoskeletal complaints because a long time a person works well no more than 8 hours a day. The longer the work time spent, the greater the likelihood of things that are not desirable, even though the results of the analysis of statistical tests that the length of work per day does not show a relationship to musculoskeletal complaints, thus that duration of work is not directly related to musculoskeletal output, but the long work process in the respondent will result in fatigue, resulting in musculoskeletal complaints.

Based on the results of the study it is known that the respondent’s working hours are erratic so that if the respondent feels tired or sick, the respondent will reduce working hours or even not work. But on the other hand, if the respondent gets a lot of passengers when working, then the respondents tend to be less rested, even though the respondents already feel tired. Based on interviews with respondents, that the income factor of passengers is a factor that can influence respondents to reduce rest, especially when competition in the Railway Station is increasingly tight, such as conventional taxis and taxis online, while the presence of online motorcycle taxi or base rent does not become competition because motorcycle taxis online only carry passengers without large capacity goods. With a lack of attention to the wife, the respondents who experienced fatigue resulted in musculoskeletal complaints. The aim is to deliver the feeder from the train station at most with the aim of the Tirtonadi bus terminal with a distance of around 700 m. Other objectives that were also frequently sent by respondents were Klewer market, Beteng market, Solo Wholesale Center (PGS) or arrived at the Surakarta Palace. Usually, after arriving at the destination, the respondent also gets a new passenger before returning to the train station. With a large number of passengers, the wife’s time is reduced and results in complaints of pain such as hand injury.
Injuries that most respondents feel the body like the wrist. A wrist injury can occur due to work such as bending the body and lifting the rear wheel of the rickshaw and holding it when the passenger rides a rickshaw and sits down. The weight of the pressure resulting from holding the load by hand results in the respondent experiencing complaints to the wrist. According to Tarwaka (2010), there are several types of musculoskeletal disorders that occur in the hands, including tendinitis, inflammation (swelling) or irritation of the tendons. It usually occurs at the point where the muscles attach to the bone. This situation will continue to develop if the continuous tendon is used to do unusual things such as strong pressure on the hand, bending the wrist while working or moving the wrist repeatedly.

Another complaint felt by respondents was a pain in the shoulder and neck. This shoulder and neck pain occur because of the position of the body when pedaling so that the rickshaw starts to run after the respondent pushes the rickshaw. Shoulder and neck pain will be increasingly felt when the respondent carries passengers or heavy items so that when the foot pedal the pedicab firmly, then the neck will feel tense due to the energy released so that the pedicab continues. The pedicab design used causes the position of the body to move away from its natural position, so pedicab drivers generally complain of upper neck, lower neck, back, right upper arm, waist, buttocks, buttocks, left forearm, right forearm, left hand, right hand, left thigh, right thigh, right knee, left calf, right calf, left foot and right foot.

Respondents’ complaints on the part of the foot most felt at the knee. This working condition occurs when pedaling a good rickshaw when pushing on a ramp, thinking of the rear wheel of a rickshaw when raising and lowering passengers, pedaling with heavy loads and carried out at a considerable distance. NIOSH (2007) states injuries to the back and knees such as - Low Back Pain due to bent back posture. If this bending posture continues it will weaken the disc and can cause disc discontinuation or herniation. At the knee is very much related to the pressure on the fluid between the bones and tendons. The pressure that occurs on the knee for a long time can cause inflammation or bursitis.

Based on the results of the study that respondents who experience pain complaints, the way to reduce pain is to rest, especially during lunch hours. Respondents sometimes sleep in passenger rickshaw seats. Fatigue can be reduced in various ways, namely by providing enough calories as input for the body, working by using good working methods, for example by working with the economic principles of movement, paying attention to the body’s ability, meaning that the expenditure of energy does not exceed income by paying attention to its limits, paying attention to regular work time by making arrangements for working hours, rest periods and facilities, during holidays and recreation and others, and trying to reduce monotony and work-related tension. The incidence of shirt injury is caused by exposure with awkward postures and the burden raised exceeds the capacity of the worker itself.

**Conclusion**

a. A large number of respondents are > 35 years old, over 7 years of working time, length of work above 8 hours per day and the average distance above 5 km.

b. Most respondents had fatigue.

c. Most respondents had complaints of the musculoskeletal system.

d. There was a correlation between the level of fatigue and complaints of the musculoskeletal system in pedicab drivers in the pedicab community, Solo Balapan Station.

**Conflicts of Interest:** All authors have no conflicts of interest to declare.

**Source of Funding:** The source of this research costs from self.

**Ethical Clearance:** The study was approved by the Institutional Ethical Board of Faculty of Public Health, Airlangga University.

All subjects were fully informed about the procedures and objectives of this study each subject prior to the study signed an informed consent form.

**References**


A Selective Membrane Electrode for Iodide Ion based on New Ionophore and its Application to Pharmaceutical Samples

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Abstract

Background and Objectives: On the basis of newly synthesized i.e. imidazolidine-2tion (IMT), highly selective poly vinyl chloride (PVC) membrane electrode was utilized as a carrier for iodide selective electrode via the incorporation of membrane ingredients within the graphite electrode surface.

Method: The impact of different factors consisting of the pH, membrane arrangement and likely intervening anions were examined on the electrode’s response characteristics. The created sensor displayed Nernstian responses to iodide across a concentration of 1× 10⁻⁶ to 0.1 M with slopes of 57.8± 0.7 mV per iodide concentration decade and 7 × 10⁻⁷M detection limit, across a pH range of 3.0-10.5.

Results: The sensor response time was 6 seconds and may be used for a minimum of 2 months with no significant divergence at potential responses.

Conclusion: The electrode was implemented with success to determine iodide within synthetic pharmaceutical samples as well as using as an indicator electrode for precipitation titration.

Keywords: Iodide-selective electrode, potentiometric sensor, imidazolidine-2tion, coated-graphite electrode.

Introduction

Iodide is a fundamental component of thyroid hormones which has a significant role in developing cell growth and brain function and its absence entails considerable delays in neurological development¹. Iodine is vital to determine iodide in natural waters and biological samples in regard to environmental and biological terms². Several analytical approaches have been conducted to determine iodide at low concentration levels since this bio-essential lement typically takes place at extremely low concentrations. The majority of these approaches need costly instruments, complex techniques, inadequate sensitivity and are time consuming e.g. classical spectrophotometry or titrimetry, and/or sample pretreatments namely in a complex matrix such as wastewater. A simple approach i.e. potentiometric detection on the basis of an ion-selective electrode has numerous advantages such as a speedy process, straightforward preparation, simple instrumentation, expeditious response, extensive dynamic range, appropriate selectivity and is cost-effective. Such properties have unavoidably produced sensors for numerous species with the available electrodes list expanding significantly over the past few years. Different types of electrodes have been recommended to determine the analyst ion. In addition to traditionally produced electrodes, the coated wire electrode has gained attention due to its simple manipulation and fabrication, great dynamic range, low detection limit and improved miniaturization potential. Thus, there has been growing interest in developing and applying potentiometric sensors. The majority of lately reported potentiometric sensors are carrier based ion-selective electrodes (ISEs) that likely act on chemical recognition principles. Numerous recognition elements types have been used in selecting or synthesizing appropriate carriers to construct ISE’s such as the utilization of adequate sized...
A selective interaction among the ionophores and anions is needed in ion selective electrodes for the purpose of successfully complex anions within a selective manner. The attraction between active sites used in anions and membranes may be separated into two categories; a quaternary ammonium and phosphonium salts by the ion exchange procedure and generates a selectivity pattern named the Hofmeister pattern. In this study, the selectivity for various ions and the impact of the membrane matrix, ionophore concentration, additives and the pH on potentiometric response characteristics regarding the suggested electrodes is examined.

**Experimental**

Coated-graphite electrodes were prepared based on a prior method. The spectroscopic grade graphite was used to prepare the graphite rods of 3mm diameter and 10 mm length. A silver loaded epoxy resin was used to glue a shielded copper wire to one of the ends of the graphite rod. The rod was then placed into the end of the PVC tube. A polishing cloth was used to polish the electrode. Water and methanol were used to rinse the electrode before being left to dry. A concoction of the membrane additive, MTOAC, PVC, plasticizer with an overall mass of 100mg was disintegrated in approximately 3 ml of THF. The electro active material imidazolidine-2tion was added to the mixture prior to being mixed. By repeatedly dipping the polished graphite electrodes into the membrane solution, the electrodes were coated. On the graphite surface, a membrane was created which was left to set overnight. Water was used to rinse the electrodes prior to being conditioned for 14 hour and 18 hour time periods in a 0.05 M potassium iodide solution for the imidazolidine-2tion. If the coating solutions are preserved in a refrigerator, they can remain stable and may be used to construct new membranes.

**Findings**

**Membrane composition effect**

As with prior reports, a plasticizer: PVC ratio, 2:1 was maintained as constant in the optimization of the suggested iodide ion-selective electrode ingredients. Because of the plasticizer effect and its quantity on the dielectric constant regarding the ligands mobility and membrane phase, it is assumed to have a significant role in detection limit control and sensitivity/selectivity of the electrode. Since membranes possess 60–70 wt.% of a plasticizer, it is predicted that the dielectric constant values of the liquid membranes are identical to the values resulting from the pure liquid plasticizer. The binding of synchronizing anions namely the thiocyanate and iodide with the complexes’ metal centers is predicted to be strengthened while the polarity of the solvents is decreased. The membrane solvent that is less polar is more appropriate for the anti-Hofmeister actions of a provided anion ionophore. Hence, at plasticizer: PVC ratio of ≈ 2 the impact of the plasticizer type concerning the response properties of the iodide ion-selective electrode was examined using 6 plasticizers with various polarities that included BA, NPOE, DMS, DOP and DBP. From 5 various implemented plasticizers, the membrane was prepared using DOP (ε=5.1) had the most favorable characteristic response. When DOP (ε= 5.1) was replaced with DBP (ε= 6.4), BA (ε= 5.0), or DBS (ε= 5.4), the electrode’s slope was reduced. For the polar membranes based on o-NPOE (ε= 23.9), the detection limit was higher. The o-NPOE polarity is higher than the polarity of DOP but they are of similar lipophilicity. This may be because of the synergism among the polarity and lipophilicity where the most beneficial results were acquired when these properties achieved an intermediate value. The electrode with DBP and DOP with low polarity compounds between the plasticizers were examined and presented useful circumstances to incorporate highly lipophile iodide ion within the membrane before coordinating it with soft mercury ion within the complex. This is in accordance with the literature that proves sensors for iodide ion-selective electrodes with comparatively non-polar membrane material may enhance iodide responses. The responses of the electrode prepared using various IMT amounts were increased when IMT concentrations were increased to 9.7. Adding more ionophores concentrations deteriorated the electrode response which was likely because of membrane saturation or membrane non-uniformity. It is established that lipophilic salts improve the response behavior and selectivity whilst reducing sample anion interference in addition to reducing membrane resistance. Neutral carrier based anion selective electrodes need lipophilic cathionic sites. However, concerning charged carrier-based ISEs, the ionic sites should hold the same charge as analyte ion. The effect of the membrane additives concentration and type were examined by integrating NaTPB or MTOAC into the membranes. The carrier-based membranes potentiometric response significantly enhanced the existence of lipophilic cationic additive,
MTOAC in comparison to the membranes with no additive. However, no responses were seen when NaTPB was integrated into the membranes. The MTOAC concentration influence on the membranes was examined at numerous additive/carrier mole ratios. The electrode with MTOAC/carrier mole with 0.42 ratio exhibited close-Nernstian response within an extensive iodide concentration range.

Response Characteristics and Selectivity of the Electrode

The results shown in Fig. 1, the potential response of the electrode based on carrier is independent of pH over the range 3.0–10.5. Dynamic response time is an important factor for an iodide sensitive electrode. In this study, the practical response time was recorded by changing solution with different low-to-high I⁻ concentrations. The actual potential versus time traces is shown in Fig. 2. As can be seen, in whole the concentration range, the electrode reaches the equilibrium response in a very short time (approx. 6 s). To evaluate the reversibility of the electrode, a similar procedure at the opposite direction was adopted. This time, measurements were performed in the sequence of high-to-low sample concentrations and the results are shown in Fig. 2, which shows that the potentiometric response of the sensor was reversible, although the time needed to reach equilibrium values were somewhat longer than that of the low-to-high order of sample concentration. Optimum conditioning time for the membrane sensor in a 5.0 × 10⁻² M potassium iodide solution is 14 h in 0.05 M potassium iodide. It then generates stable potentials when placed in contact with I⁻ solutions. The long-term stability of the electrode were studied by direct periodically recalibrating in standard solutions (KI and air and distilled water) and calculating the response slope over the range of 1.0 × 10⁻⁵ to 1.0 × 10⁻¹ iodide ion. The slopes of the electrode responses were reproducible to within 1.0 mV/decade over a period of 2 months, but life time studies for more than this period have not been done. Therefore, the membrane electrode can be used for at least 2 months, without a considerable change in the response characteristics towards iodide ion. The electrode was stored in solution when not in use. The potentiometric response of the electrode was examined in the concentration range 1.0 × 10⁻⁷–1.0 × 10⁻¹ M. The calibration plots for iodide selective electrode are shown in Fig. 3, which show linearity over the concentration range of 1.0 × 10⁻⁶–1.0 × 10⁻¹ M and with a detection limit of ≈7.0×10⁻⁷ M and sensitivity of 55.2 ±0.9 mV/decade of iodide concentration (n=6).
Figure 3. Application of the electrode based on IMT for potentiometric titration of (A) 100 ml $1 \times 10^{-3}$ M Ag$^+$ with 0.1 M I$^-$ and (B) 100 ml $1 \times 10^{-3}$ M I$^-$ with 0.1 M Ag$^+$.

**Table 1. Specification of iodide selective electrodes**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrode type</td>
<td>Coated–graphite electrode</td>
</tr>
<tr>
<td>MTOAC /Carrier ratio</td>
<td>0.42</td>
</tr>
<tr>
<td>pH range</td>
<td>3 –10.5</td>
</tr>
<tr>
<td>‘Conditioning time</td>
<td>At least 18 h in 0.05 M KI</td>
</tr>
<tr>
<td>Linear range (I$^-$, M)</td>
<td>$1 \times 10^{-6}$ - $1 \times 10^{-1}$</td>
</tr>
<tr>
<td>Slope (mV/decade)</td>
<td>55.2</td>
</tr>
<tr>
<td>Detection limit (M)</td>
<td>$\sim 6 \times 10^{-7}$</td>
</tr>
<tr>
<td>Standard deviation of slope (mV/decade)</td>
<td>± 0.9</td>
</tr>
<tr>
<td>Standard deviation of measurement</td>
<td>± 0.4 at $1 \times 10^{-2}$ M</td>
</tr>
<tr>
<td></td>
<td>± 0.8 at $1 \times 10^{-3}$ M</td>
</tr>
<tr>
<td>Response time (s)</td>
<td>≤ 6</td>
</tr>
<tr>
<td>Life time of the electrode</td>
<td>At least 3 months</td>
</tr>
</tbody>
</table>

**Selectivity of the Electrode**

The selectivity behavior is obviously one of the most important characteristics of an ion-selective electrode, determining whether a reliable measurement in the target sample is possible. In order to assess the selectivity of the proposed iodide ion selective electrode over other anions the method of fix interference method [FIM] and separate solution method [SSM] was employed.$^{14,15}$ According to this method, the potentiometric selectivity coefficients, $K_{pot}$ I, can be evaluated from the potential measurements on solutions containing a fixed concentration (0.01 M or 0.001 M) of interfering ion (respective potassium or sodium salts) and varying concentration of iodide ion in FIM and in SSM potential of different solution containing same amount of iodide or interfering ion (0.01 M). As can be seen, the electrode based on carrier has not shown the tendency toward the highly lipophilic anions such as ClO$_4^-$, N$_3^-$, Br$^-$, NO$_3^-$, and NO$_2^-$.

**Indirect Determination of Iodine-containing Drug**

The proposed electrode was applied for the indirect determination of iodine containing drugs including iodiquinol (Jalinous Pharmaceutical Co, Tehran, Iran) and Levothyroxin (Iran Hormon Co, Tehran, Iran) as follow: 2 g of sodium were placed into an alkaline fusion tube and heated until all of the sodium was fused. Then an appropriate and accurately weighed amount of finely powder tablet (i.e., 0.1 g of 4 mixture tablet) was added to hot tube, and the resulting mixture was completely burned. The hot tube was immediately transferred into a beaker containing 25 ml distilled water. After cooling the reaction mixture was filtered and washed with water and diluted to mark in a 50 ml calibrated volumetric flask. The iodide released from the decomposition of drug was determined by potentiometric titration method using standardized silver nitrate, the proposed electrode as indicator electrode.

**Direct Determination of Iodide-containing Drug**

The resulting electrodes were applied to determine iodide in a drug preparation (Meglumine Compound Injection, from Darou Pakhsh Pharmaceutical Co., Iran; is an iodide containing drug used for protection from light and secondary X-rays). The sample was prepared by refluxing 1.0 ml of the drug preparation in concentrated sodium hydroxide solution in the presence of zinc powder for 30 min.$^{16}$ After cooling, the reaction
mixture was filtered and washed with water three times. The filtrate was acidified with H$_2$SO$_4$ and diluted to 1 with water. The iodide content of the resulting solution was determined potentiometrically by the standard addition method.

**Conclusions**

This iodide selective electrode based on IMT as new carrier can be used for determination of iodide in polluted and industrial samples. Due to the high mechanical resistance and durability of the coated-wire electrode, and low solubility of the carrier used, the proposed electrode can be used in flowing streams. The characteristics and the typical applications presented in this paper, make the electrode suitable for measuring the iodide content in a wide variety of samples, without a significant interaction from concomitant anionic species. The results show that there was a coordination interaction between iodide and the proposed carrier, which played an important role in the response characteristics and selectivity of the electrode.

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**Conflict of Interest:** Authors have declared that no competing interests exist.

**Ethical Clearance:** Ethical clearance taken from ethical committee of Abadan Faculty of Medical Sciences.

**References**


Rituximab in the Treatment of Refractory Myasthenia Gravis: Studying the Outcomes using MMT Score and Need for Plasmapheresis in Baghdad Teaching Hospital

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Abstract

Introduction: Myasthenia gravis (MG) is an uncommon autoimmune disease that is caused by acetylcholine receptor antibodies (AChRA) at the neuromuscular junction. Its pathogenesis involves complement fixing antibodies directed against acetylcholine receptors, muscle-specific tyrosine kinase or low density lipoprotein receptor–related protein.

Method: A descriptive case series study investigating rituximab in refractory MG was conducted in Baghdad teaching hospital. Patients who were included in the study had received a regimen of rituximab that was deemed appropriate by their treating physician.

Results: A total of 24 patients with mean disease duration of 6.3 years ±3.6 standard deviation were included in this study. The results showed that the average age of patients was 33.3 years ±10.1 SD and 62.5% was females. All twenty four patients showed an obvious improvement in clinical status after finishing the follow-up period. The results demonstrated from all patients that enrolled in our study, only one patient that who was on 14 sessions of plasmapheresis was in need for plasmapheresis after induction of rituximab and this difference was statistically significant.

Conclusion: There were a great clinical improvement as shown with MMT score in addition to discontinuation of plasma exchange treatments following treatment with rituximab.

Keywords: Rituximab, Myasthenia gravis, Refractory, plasmapheresis
review of population-based studies, and showed a higher incidence rate of MG in young women and older men\textsuperscript{[9]}. The neuromuscular abnormalities in MG are thought to be an autoimmune response related to specific anti-AChR antibodies, but how the autoimmune response is started and preserved in MG is not fully understood\textsuperscript{[10]}. AChR antibodies mostly belong to the IgG1 and IgG3 subclasses, which stimulate the complement cascade to destruct the postsynaptic membrane, which may lead to up regulation of inflammatory cytokines\textsuperscript{[11]}. The ultimate goal of treatment is to achieve complete stable remission, defined as no myasthenic symptoms or signs without any ongoing treatment for at least 1 year\textsuperscript{[12]}. Therapies in MG should therefore eliminate patients’ symptoms within the boundaries of adverse events associated with treatment itself\textsuperscript{[13]}.

**Patients and Method**

A descriptive case series study examining rituximab in refractory MG was performed in Baghdad teaching hospital. Patients who were included in the study had received a regimen of rituximab that was deemed appropriate by their treating physician. Total of twenty four patients identified with refractory generalized MG were enrolled in the study. Physical tests were estimated before and after treatment with rituximab. MMT score is used to show clinical improvement. MMT is a procedure for the evaluation of strength of individual muscle or muscles group based upon the effective performance of movement relation to the forces of gravity or manual resistance.

There were no specific criteria used to stage clinical response against no clinical response. The main outcome of the study was the improvement in MMT score and symptoms of patients, with the other outcomes being the change in the frequency of plasma exchange which were done at the estimation of the clinician. Patients that are included in the study are 13 to 90 years old that must have refractory MG, that not respond to glucorticoid and other immunosuppressive therapy. Subjects must be on a stable standard immunosuppressive regimen with no history of thymoma, tumor, infection, or interstitial lung disease on chest CT, MRI, or chest x-ray. Patients that are excluded from the study are patients with history of chronic degenerative, psychiatric, or neurologic disorder other than MG that can produce weakness or fatigue. Female subjects who are premenopausal who are pregnant, breast feeding or not use effective method for contraception. Protocol for Rituximab was administered in a standard dose of 1g. Every cycle is estimated as one infusion for two weeks. The space between cycles was adjusted as 6 months.

To see the safety and adverse events, we collected notes from the infusion center and also, a complete blood count and, liver function test profiles present in patient’s medical records. SPSS version 22 was used for data entry and analysis. Mean and standard deviation was used to represent the numerical data while the frequency and percentage for categorical data. Independent student T test and the test chi-square (Fischer exact test if not applicable), were used to confirm significance \( p < 0.05 \) was considered significant.

**Results**

A total of 24 patients with mean disease duration of 6.3 years ±3.6 SD were included in this study. The results showed that the mean age of patients was 33.3 years ±10.1 SD and 62.5% was females. All twenty four patients showed a marked improvement in clinical status by the end of the follow-up period. Five of the patients underwent a single cycle of rituximab, two received two cycles of rituximab while the remaining received either three or more cycles of rituximab. The results indicated there was no significant difference (\( p = 0.5 \)) in plasmapheresis session that as needed after induction of rituximab when compared according to number of cycle of rituximab in term of < 4 or \( \geq 4 \) cycles, where the results showed that only one patient was in need for plasmapheresis session of those who received less than 4 cycles of rituximab while no one in need for plasmapheresis session of those who received more than 4 cycles of rituximab as seen in table 1.
The results demonstrated from all patients that enrolled in our study, only one patient that who was on 14 sessions of plasmapheresis was in need for plasmapheresis after induction of rituximab and this difference was statistically significant (p=0.03) as seen in table 2.

Table 1: Association between no. of cycles of treatment and number of pl. exchange session

<table>
<thead>
<tr>
<th>Cycle of treatment with rituximab</th>
<th>Plasmapheresis session</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>No. %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;4</td>
<td>1</td>
<td>7.1%</td>
</tr>
<tr>
<td>≥4</td>
<td>0</td>
<td>0.0%</td>
</tr>
</tbody>
</table>

Table 2: Associations between plasmapheresis sessions pre and post treatment with rituximab

<table>
<thead>
<tr>
<th>Plasmapheresis session-pre rituximab/no. of patients</th>
<th>Plasmapheresis session/post rituximab</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Count</td>
<td>Row N %</td>
<td>Count</td>
</tr>
<tr>
<td>No sessions(1 patients)</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>1 session(1 patients)</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>2 session(2 patients)</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>3 session(5 patients)</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>4 session(6 patients)</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>5 session(3 patients)</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>6 session(1 patients)</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>7 session(1 patients)</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>8 session(1 patients)</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>10 session(1 patients)</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>12 session(1 patients)</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>14 session(1 patients)</td>
<td>1</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Regarding MMT score the results indicated that the highest frequency (50%) of patients was in score 2 of MMT score before using the rituximab followed by score 3(33.3%) and lastly the score 1(16.7%) as seen in table 3.
Table 3. Frequency of patients according to MMT scores before using rituximab.

<table>
<thead>
<tr>
<th>MMT Score</th>
<th>No. of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score-2</td>
<td>12</td>
<td>50.0</td>
</tr>
<tr>
<td>Score-3</td>
<td>8</td>
<td>33.3</td>
</tr>
<tr>
<td>Score-1</td>
<td>4</td>
<td>16.7</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>100.0</td>
</tr>
</tbody>
</table>

After using the rituximab, the highest frequency of patients 12(50%) was improved to score 5 of MMT followed by score 4 (33.3%) and only 16.7% was in score 3 as seen in table 4.

Table 4. Frequency of patients according to MMT scores post using rituximab.

<table>
<thead>
<tr>
<th>MMT Score</th>
<th>No. of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score-5</td>
<td>12</td>
<td>50.0</td>
</tr>
<tr>
<td>Score-4</td>
<td>8</td>
<td>33.3</td>
</tr>
<tr>
<td>Score-3</td>
<td>4</td>
<td>16.7</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>100.0</td>
</tr>
</tbody>
</table>

The finding revealed that from 4 patients who was with score one before treatment with rituximab; 3 patients were improved to score 4 and 1 to score 5. From 12 patients who were in score 2 before using of rituximab; 4 patients were improved to score 3, 3 patients improved to score 4 and 5 patients to score 5. From 8 patients who were in score 3 before treatment with rituximab; 2 patients were improved to score 4 and 6 patients to score 5. So the results revealed that the frequency of patient who had pretreatment high score of MMT, who were reach the highest score after treatment with rituximab was higher than those who were already had low score before using the rituximab but this difference was non-significant as seen in table 5.

Table 5: Association of MMT pre and post using of rituximab

<table>
<thead>
<tr>
<th>MMT/before</th>
<th>MMT/after</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score-3</td>
<td>Score-4</td>
<td>Score-5</td>
</tr>
<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Score-1(n=4)</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Score-2(n=12)</td>
<td>4</td>
<td>33.3%</td>
</tr>
<tr>
<td>Score-3(n=8)</td>
<td>0</td>
<td>0.0%</td>
</tr>
</tbody>
</table>
Discussion

In this study of 24 patients that present with refractory generalized myasthenia gravis, we see that rituximab result in a sustained clinical improvement as demonstrated by improved MMT score in addition to reduce frequency or lack of need for plasma exchange treatments as shown with Anderson et al study[14]. At time of this study, all patients were followed for 1 year following infusion with rituximab. The results of this analysis favor the theory that rituximab can be effective for treatment of refractory MG which is compatible with small reports that were done previously as in Zebardast et al and other studies[15,16]. Most patients may need two or more cycles of rituximab for obvious decrease and discontinuation of other immunosuppressant, in addition to the attainment of disease remission. As there is no fixed protocol for rituximab use in myasthenia gravis, retreatment is decided based on disease activity and clinical improvement, with a minimum interval between infusions of 6 months which is similar to Peres et al[17]. A perfect protocol has not yet been confirmed, however, some studies used peripheral B-cell count as a guide for retreatment with rituximab and to decrease toxicity and side effects [18,19]. An additional studies are required to recognize the best objective clinical indicators and to fix pharmacokinetics in this kind of patients. Immunoglobulin levels, B-cell counts, titers for antibody would seem the most helpful objectives at this time but in our study we depend on symptoms improvement and MMT score. All patients in this study can tolerate rituximab with no severe hematologic derangements[20,21]. The patients in this study were monitored clinically for rituximab adverse effects while in the infusion center as well as with CBC and LFT at baseline and after each infusion. All patients in this study can tolerate rituximab with no severe hematologic derangements. Infusion reaction is the most common side effect reported in general[22]. There is a need for a larger prospective controlled trial to gain more definitive conclusions about the efficacy of rituximab in the treatment of refractory MG. The strong effect of rituximab in patients with refractory MG in our center as well as in similar studies is promising and suggests that further investigation of this agent in MG is warranted[23,24].

Conclusion

There were a great clinical improvement based on MMT score, in addition to decrease or cutting the use of plasma exchange following treatment with rituximab. Rituximab is an attractive treatment because of its mechanism of action that result in targeting CD20-positive B cells which are engaged in the production of antibodies. Most patients in this study can tolerate rituximab with no severe hematologic derangements and mild infusion reactions were the most common side effects associated with our study.

Conflict of Interest: Nil

Source of Funding: self

Ethical Clearance: Ethical Clearance: verbal consents were obtained from the all patients before their enrollment in the study.

References


Assessment of Nurses’ Knowledge toward Nursing Intervention for Eye Trauma at Baghdad Teaching Hospitals

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Abstract: Nurses in emergency care for eye trauma usually concentrate on life threatening issues which are emergency in most situations that lead to little concern of the less emergency issues like eye problems even it is serious problem. Objectives: Assessment of Nurses’ Knowledge toward Nursing Intervention for Eye Trauma and to find out a relationship between nurses knowledge with some variables such as (educational level, year of experience in hospital, training course). Methodology: A descriptive study was conducted in the period of December 10th 2017 up to the end of 5th May 2018. The sample consisted of (100) nurses who were systematically selected one by one. The data collected was analyzed using SPSS version 17.0. Results: The majority of the study were female who accounted for (59%) of the total participants while male constituted (41%). Most of the study participants (32%) were ages over 41 years old. (43%) of the nurses were Nursing high school graduate. Seventy eight percent of the nurses were married and fourth eight percent were barely sufficient. Majority (83%) of nurses had training course. Conclusions: Findings of study demonstrate that, nurses have good knowledge about eyes Trauma.

Keywords: Knowledge, intervention, nurses, Eye Injuries

Introduction

Eye injuries come at a high cost to society and can be avoided. Primary explosion injuries can be initial, from the same explosion wave secondary from the wind-blown fragments. Post-secondary; due to structural collapse or thrown against a fixed body; or the quartet, burns and injuries of indirect[1]. Chemical eye injuries are common and represent one of the “true” eye emergencies. Virtually any chemical can cause ocular irritation. Most of these injuries are unpredictable and do not cause permanent lesions (e.g., shampoos, defense sprays, home cleaning solutions, etc.) while others may result in permanent decay [2]. Eye trauma is the main cause of impaired vision and affects the individual by influencing their quality of life and society by causing loss of ability to work [3]. Penetrating orbital injuries pose a serious threat to vision, eye movement, and life in some cases. Previous studies have shown that risk and type of infection are often associated with age, sex and race [4]. Management of an injured eye requires taking accurate history, evaluation of vision that measures the acuity and if there is a relative glaucoma defect as well as careful examination of the eyes, under an aesthetic if necessary. A lateral canthotomy with cantholysis should be performed immediately if there is a sight-threatening retro bulbar haemorrhage. Systemic antibiotics should be prescribed if there is suspected penetration or perforation of injury. The world should be protected by ruptured eye shield [5].

Materials and Method

A descriptive study: Assessment of Nurses’ Knowledge toward Nursing Intervention for Eye Trauma at Baghdad Teaching Hospitals. This study was conducted at Baghdad Teaching Hospitals between December 10th 2017 up to the end of 5th May 2018. A tool of knowledge questionnaire was developed and distributed to the participants in this study. The sample consisted of (100) nurses at Baghdad Teaching Hospital, Ibn-AL-Hathaim Teaching Hospital, AL.Yarmouk Teaching Hospital, Imamiein Kadhimein Medical City. A questionnaire- interview format was designed and developed by the researcher for the purpose of the study; such development was employed through the available literature, clinical background and interview with nurses. All the items were measured on scale of (2) indicates that the know 1; don’t know 0.The questionnaire consisted of (2) parts. Part I: Demographic Information Sheet. Part II: Assessment of Nurses knowledge toward Nursing Intervention for Eye Trauma which Includes:
foreign body, chemical substance and embedded object. Rating scale was used to rate the frequency and extension of the problems. The content validity of the instrument was established through a panel of (10) experts. Test- retest reliability was determined through a computation of person correlations for the scales. The data were collected by using the questionnaire structured format through interview and inspection technique. The determination was conducted during the period from 1st January 2018 to 30th February 2018. The data were analyzed through descriptive data analysis and inferential data analysis the data were analyzed through the use of Statistical Package of Social Sciences (SPSS) version (17).

**Results**

**Table (1): The Mean of Score Nurses knowledge toward Eye Trauma Items.**

<table>
<thead>
<tr>
<th>No</th>
<th>Items</th>
<th>Know</th>
<th>Don’t know</th>
<th>SD</th>
<th>MS</th>
<th>A.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Foreign body</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>To Attempt to flush and foreign objects from the eye whilst keeping the injures eye down</td>
<td>22</td>
<td>78</td>
<td>.416</td>
<td>.22</td>
<td>poor</td>
</tr>
<tr>
<td>2</td>
<td>keep the person still and comfortable</td>
<td>98</td>
<td>2</td>
<td>.141</td>
<td>.98</td>
<td>Good</td>
</tr>
<tr>
<td>3</td>
<td>Place a sterile pad over the eye.</td>
<td>96</td>
<td>4</td>
<td>.197</td>
<td>.96</td>
<td>Good</td>
</tr>
<tr>
<td>4</td>
<td>Avoid putting any pressure on the eye</td>
<td>92</td>
<td>8</td>
<td>.273</td>
<td>.92</td>
<td>Good</td>
</tr>
<tr>
<td>5</td>
<td>Encourage the injured person not to blink or move either eye as movement of the unaffected eye will also cause movement of the injured eye</td>
<td>76</td>
<td>24</td>
<td>.429</td>
<td>.76</td>
<td>Good</td>
</tr>
<tr>
<td>6</td>
<td>If possible the victim should keep the uninjured eye closed or covered</td>
<td>70</td>
<td>30</td>
<td>.461</td>
<td>.70</td>
<td>Good</td>
</tr>
<tr>
<td>7</td>
<td>Seek medical advice</td>
<td>94</td>
<td>6</td>
<td>.239</td>
<td>.94</td>
<td>Good</td>
</tr>
<tr>
<td>8</td>
<td>Do not place any objects</td>
<td>94</td>
<td>6</td>
<td>.239</td>
<td>.94</td>
<td>Good</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>642</td>
<td>158</td>
<td>.299</td>
<td>.80</td>
<td>Good</td>
</tr>
<tr>
<td>B</td>
<td>Chemical substances</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Immediate copious irrigation with a minimum of 1-2 L of normal saline</td>
<td>84</td>
<td>16</td>
<td>.368</td>
<td>.84</td>
<td>Good</td>
</tr>
<tr>
<td>2</td>
<td>Instill a topical anesthetic</td>
<td>68</td>
<td>32</td>
<td>.469</td>
<td>.68</td>
<td>Good</td>
</tr>
<tr>
<td>3</td>
<td>Use eyelid retractor</td>
<td>76</td>
<td>24</td>
<td>.429</td>
<td>.76</td>
<td>Good</td>
</tr>
<tr>
<td>4</td>
<td>Double eversion of the eyelids</td>
<td>64</td>
<td>36</td>
<td>.482</td>
<td>.64</td>
<td>Good</td>
</tr>
<tr>
<td>5</td>
<td>No corneal involvement -ATB + steroid eye drop</td>
<td>68</td>
<td>32</td>
<td>.469</td>
<td>.68</td>
<td>Good</td>
</tr>
<tr>
<td>6</td>
<td>Going to ophthalmologist</td>
<td>94</td>
<td>6</td>
<td>.239</td>
<td>.94</td>
<td>Good</td>
</tr>
<tr>
<td>7</td>
<td>Preservative-free artificial tears</td>
<td>60</td>
<td>40</td>
<td>.492</td>
<td>.60</td>
<td>Good</td>
</tr>
<tr>
<td>8</td>
<td>Instill topical cycloplegic and topical antibiotic</td>
<td>58</td>
<td>42</td>
<td>.496</td>
<td>.58</td>
<td>Good</td>
</tr>
<tr>
<td>9</td>
<td>Taking oral analgesic</td>
<td>78</td>
<td>22</td>
<td>.416</td>
<td>.78</td>
<td>Good</td>
</tr>
<tr>
<td>10</td>
<td>Pressure patch or bandage on the contact lens</td>
<td>46</td>
<td>52</td>
<td>.502</td>
<td>.48</td>
<td>Poor</td>
</tr>
<tr>
<td>11</td>
<td>Use the Anatiglaucoma</td>
<td>28</td>
<td>72</td>
<td>.451</td>
<td>.28</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>640</td>
<td>358</td>
<td>.404</td>
<td>.58</td>
<td>Good</td>
</tr>
<tr>
<td>C</td>
<td>Embedded object</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Do not try to remove the object from the eye</td>
<td>90</td>
<td>10</td>
<td>.301</td>
<td>.90</td>
<td>Good</td>
</tr>
<tr>
<td>2</td>
<td>Try to place a protective cover around and over the injured eye without pressure</td>
<td>86</td>
<td>14</td>
<td>.349</td>
<td>.86</td>
<td>Good</td>
</tr>
<tr>
<td>3</td>
<td>Ensure that the pressure on the protective cover of the infected eye</td>
<td>88</td>
<td>12</td>
<td>.327</td>
<td>.88</td>
<td>Good</td>
</tr>
<tr>
<td>4</td>
<td>Place a pad over the uninjured eye to minimize the movement in both eyes</td>
<td>32</td>
<td>68</td>
<td>.469</td>
<td>.32</td>
<td>Poor</td>
</tr>
<tr>
<td>5</td>
<td>Stop examination</td>
<td>68</td>
<td>32</td>
<td>.469</td>
<td>.68</td>
<td>Good</td>
</tr>
<tr>
<td>6</td>
<td>Give tetanus prophylaxis</td>
<td>56</td>
<td>44</td>
<td>.499</td>
<td>.56</td>
<td>Good</td>
</tr>
<tr>
<td>7</td>
<td>Not taking antibiotics by mouth</td>
<td>50</td>
<td>50</td>
<td>.503</td>
<td>.50</td>
<td>Good</td>
</tr>
<tr>
<td>8</td>
<td>Refer immediately to ophthalmologist</td>
<td>90</td>
<td>10</td>
<td>.302</td>
<td>.90</td>
<td>Good</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>560</td>
<td>240</td>
<td>.402</td>
<td>.70</td>
<td>Good</td>
</tr>
</tbody>
</table>

A.D.): Assessment Degree, M.s=mean of score [(0 - .49) = poor (F); (0.5 – 1) = good(P)]
This table shows the total nurses’ knowledge toward nursing intervention for eye trauma, which indicated that nurses had good knowledge toward for eye trauma, with respect to the total mean of score (MS) which was (.80) for foreign body domain, MS (.58) for chemical substances domain and MS (.70) for embedded object.

Table (2): Association between (Level of Education, Experience of nursing in Hospitals, Training Course) and Nurses Knowledge.

<table>
<thead>
<tr>
<th>Nurses knowledge Level of education</th>
<th>Poor</th>
<th>Good</th>
<th>Total</th>
<th>$\chi^2$-obs.</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nursing high school graduate</td>
<td>4</td>
<td>39</td>
<td>43</td>
<td>3.550a</td>
<td>NS</td>
</tr>
<tr>
<td>Institute graduate</td>
<td>2</td>
<td>36</td>
<td>38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>College of Nursing</td>
<td>4</td>
<td>15</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>90</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$\chi_2$ criti. = 5.99 df = 2 $p \leq 0.05$

<table>
<thead>
<tr>
<th>Nurses knowledge Experience of nursing</th>
<th>Poor</th>
<th>Good</th>
<th>Total</th>
<th>$\chi^2$-obs.</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-5</td>
<td>6</td>
<td>21</td>
<td>27</td>
<td>7.223a</td>
<td>NS</td>
</tr>
<tr>
<td>6-10</td>
<td>0</td>
<td>22</td>
<td>22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-15</td>
<td>2</td>
<td>21</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;16</td>
<td>2</td>
<td>26</td>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>90</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$\chi_2$ criti. = 7.815 df = 3 $p \leq 0.05$

<table>
<thead>
<tr>
<th>Nurses knowledge Training course</th>
<th>Poor</th>
<th>Good</th>
<th>Total</th>
<th>$\chi^2$-obs.</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>4</td>
<td>79</td>
<td>83</td>
<td>14.560a</td>
<td>H.S</td>
</tr>
<tr>
<td>No</td>
<td>6</td>
<td>11</td>
<td>17</td>
<td></td>
<td></td>
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<tr>
<td>Total</td>
<td>10</td>
<td>90</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$\chi_2$ criti. = 3.841 df = 1 $p \leq 0.05$

*: $P \leq 0.05$; **: $P \leq 0.01$, $\chi^2$, t-test

This table indicates that there is no significant association between levels of education, experience of nursing in hospitals with nurse’s knowledge and there is significant association between training courses with nurse’s knowledge.
Discussion

Through the course of the data analysis of the present study the finding showed that the majority (59%) of the study were female while the remaining were male. The highest percentage of age group in present study (43%) were over 41 years old and lowest percentage(8%) were (21- 25) years old. Concerning marital status and level of education, (78%) of the sample were married, most of them are (43%) nursing had high school graduate. monthly income for study sample were (48%) o barely sufficient income.

These results are agreement with the findings obtained from other study, who shows that nurses in age group of 41–60 had good knowledge (11.1%) compared to age group of 20–30 (1.1%), also this study disagree with level of education, who shows nurses with certificates have good knowledge (25%) compared to post basic nursing (0%) [6].

Regarding experience years, majority (28%) of the study was experience years in nursing and (37%) of nurses had Experience years in eyes hospitals, (83%) have training and (55%) had number of courses in nursing.

These results are accordance with findings obtained from other study, shows that after the training course, 47.5% of the respondents were somehow familiar with high reliability organizations model (HROs), and 52.5% admitted that they were completely familiar with this model, although, only 18.8% proved completely familiar with HROs model before the training course [7].

The result of accurate study that show foreign body domain in table (2) for nurses knowledge is high good knowledge in item (keep the person still and comfortable) and poor knowledge in item (To Attempt to flush and foreign objects from the eye whilst keeping the injured eye down). The mean of score is good at all Foreign body domains. A finding suggest the large injured of patients to enter foreign body to the eye hospital in the eyes that leads to frequent nurses cope with these injuries and to increase knowledge about these trauma.

This finding was similar to a study conducted by other researcher who stated that study which reported that the chemical injuries were accidental in 73.9% of the cases and of these 76.5% were work related. The remaining 26.1% resulting from assault were bilateral and led to blindness in all the patients. Main injurious agents were acid and alkali [9].

The finding demonstrated the mean of score of embedded object in table (2) for nurses knowledge are high good knowledge in item (Do not try to remove the object from the eye) and (Refer immediately to ophthalmologist) and poor in item (Place a pad over the uninjured eye to minimize the movement in both eyes). The mean of score is good at all embedded object domains. This finding indicted nurses have good knowledge to deal with the Embedded object of eyes in hospitals.

This finding are good agreement done which other researchers who reported that successful removal of penetrating oculo-cranial foreign body by non-operative methods can be done in the emergency department in carefully selected patients [10].

Level of education, experience of nursing in hospitals in comparison of the respondent’s total Nurses knowledge domain in table (4), there is non-significant association between level of education (χ² obs=3.550)a and poor knowledge in item (To Attempt to flush and foreign objects from the eye whilst keeping the injured eye down). The mean of score is good at all Foreign body domains. A finding suggest the large injured of patients to enter foreign body to the eye hospital in the eyes that leads to frequent nurses cope with these injuries and to increase knowledge about these trauma.

The result of accurate study that show foreign body domain in table (2) for nurses knowledge are high good knowledge in item (Going to ophthalmologist) and poor in items (Pressure patch or bandage on the contact lens) and (Use the Anatiglaucoma). The mean of score is good at all Foreign body domains. A result that refers to frequent patients enter the chemical injury to the eye hospital in the eyes of leads to frequent nurses to deal with chemical injuries and to increase knowledge and skills about these trauma.

Training course in comparison of the respondent’s total nurses knowledge domains with training course in

This result of study are disagreements with other studies done by other researchers whose reported that the association between knowledge and education level there was statistically significant (significant level is value less than 0.05) association between the two variables(χ² obs== 8.003 a, df=1 , p ≤ 0.05) [11].

Training course in comparison of the respondent’s total nurses knowledge domains with training course in
table (4), there is significant between training course ($\chi^2_{\text{obs}}=14.560a$, df=2, $p \leq 0.05$) with nurses knowledge domains.

These result were similar to those result obtained from other researcher who reported that total knowledge of staff about high reliability organizations model increased after training course ($P<0.0001$) [7].

**Conclusions**

This study demonstrate that, despite good nurses knowledge regarding eyes trauma, as well as study indicated overall nurses have positive correlation eyes trauma and training course.

**Recommendations**

1. Health education programs for nurses about eyes trauma.

2. Increases training course for nurses inside and outside in Iraq that contributed to improve nurses knowledge and practice about eyes trauma.

This research was funded by Author. Moreover, we would like to thank the study participants and data collectors for their fully participation and responsible data collection.

**Funding:** None

**Conflict of Interest:** None declared

**Ethical approval:** Not required

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Anti malignant (HepG2, MOLT-3) Activity of One, Two, Three- Triazoles New Prepared Bearing Hetero Compounds in Baghdad

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Abstract

In the current work, chemistry a group of related chemicals that are similar in structure or properties of new heterocyclic compounds were prepared from para nitrobenzoic acid. This work includes four parts: the first part Preparation of 5-(4-nitro phenyl)-1,3,4-thiadiazol-2-amine by the reaction of p-nitrobenzoic acid with thiosemicarbazide, the second part prepared 2-chloro-N-(5-(4-Nitrophenyl)-1,3,4-thiadiazol-2-yl) Acetamide by reaction Chloroacetyl chloride with compound and part three contain reaction compound(2) with urea/thiourea to get compounds cyclization by using urea (thiourea) finally step reaction these compounds with different aldehydes and ketones in glacial acetic acid \(^{(6-10)}\), as shown in Scheme 1

The aim of present work is synthesis new derivatives of 1,2,3-Triazole having heterocyclic compounds on ring from the reaction of azine with a different reagent to give (1,2,3- Triazole) rings. The 1,2,3-Triazoles It has been derivatives A long time ago and has been Scout mainly for their potential in anti-tumor chemotherapy the prepared of 11,2,3-Triazole financial products have good Anti malignant (HepG2, MOLT-3) Activity. Many groups have carried out focused research in this particular

Keywords: 1,2,3-Triazoles • Heterocyclic • Anti malignant• (HepG2, MOLT-3)

Introduction

Heterocyclic compounds are considered an important branch of Bioorganic compounds due to their implication in drugs and industrial studies. They are cyclic compounds in which one or more of the atoms of the ring are hetero atoms. Nitrogen, oxygen and sulfur are considered the most hetero atoms known \(^{(1, 2)}\).

Heterocyclic compounds are found as construction units through several biological molecules \(^{(3)}\), and mostly are molecules which contain five, six and seven-membered rings \(^{(4)}\).

Triazoles comprising of nitrogen containing heterocycles, fused thiazoles, benzimidazoles, lindoles, etc.

Constitute an important base in biological science and medicinal chemistry. Triazine:

The 1,2,3-Triazine, is an Aaza analogue of pyridine, and its derivatives form an important class of heteroaromatic compounds with various interesting biochemical properties.

Triazines are six-membered aromatic rings containing three nitrogen atoms; there are three possible arrangements of the nitrogen atoms in the ring:

1,2,3-Triazines are sometimes referred to as the symmetrical isomer and 1,2,4-triazines as the asymmetrical form; 1,2,3-triazines are often referred to as vic(vicinal) isomers \(^{(4-6)}\).
synthesized 3-Ssubstituted-pyrido[1,2-aa][11,3,5]triazin-2,4-dione [6] by Ccycloaddition betweenndiphenyl methylisocyanate and 2-pyridyl isocyanate according to the following reaction steps:

\[
\begin{align*}
\text{N} & \quad \text{NaNO}_2/HCl \\
\text{N} & \quad \text{NaN}_3 \\
\text{CO} & \quad \text{NHNH}_2 \\
\text{N} & \quad \text{CO} \\
\text{N} & \quad \text{NCO} \\
\text{Curtius rearrangement} & \quad \text{heat, } -\text{N}_2
\end{align*}
\]

2-pyridyl isocyanate

The expansion of new anticancer curative agents is one of the fundamental targets in medicinal chemistry.

Cytotoxicity and genotoxicity of anticancer treatment to the normal cells are major troubles in cancer therapy and engender the risk of inducing secondary malignancy.

A dose of an anticancer drug adequate to kill Tumor cells.

In contemporary years, there has been a mindful search for the invention and development of novel selective anti-cancer agents, devoid of many of the unpleasant side effects of conventional anticancer agents.

The synthesis of a newer class of anticancer agents is in need of time.

In this work prepared the anticancer compounds 1, 2, 3, triazole by linked with another active site moiety by used different delineation [10].
2. Synthetic delineation

A large number of biologically active molecules containing a motif of 1, 2, 3 triazole were well synthesized before the “clickon chemistry” approach became common. A one, two, three-Triazole ring system has been a subject of intense research\(^{(1,2,8)}\) due to its versatile potential to interact with diverse biological systems.

In recent years, many synthetic methodologies have been developed for the synthesis of this ring system. The most common reaction for the production of cracks 1,2,3 triazole is the three-dimensional load 1,3 dipolar cycloaddition also known as Huisgen cycloaddition, between an azide and a terminal alkyne, under conditions thermal but was not initially applied much in drugs synthesis owing to the poor regioselectivity.
Results & Discussion

Chemistry

The synthetic preliminary draft of a treaty or other agreement used for the process of combining different ideas, influences of the desired compounds bioactive property have been depicted in Schemes.

The structure of the newly synthesized compound [1] is in agreement with IR and ^1^H-NMR. Its IR spectrum showed clearly.

The FTIR spectrum of compound [1] revealed a medium stretching vibration band at (1660 cm^-1) that corresponds to (C=C) amide band (see Figure 1, and Table 2). In this spectrum, there are four other characteristic bands at (2952 and 3091 cm^-1), (3249, 1670 and 752 cm^-1) due to (C-H aliph., NH, C=N and C-S cm^-1) group stretching vibrations, respectively. That mean compound (1), ^1^H NMR spectra of prepared compound.

multiplet -H of aromatic rings (6.24- 8.06); Singlet 1H of –NH2 group (8.30); Singlet 1H

The FTIR spectrum of compounds [2] have important characteristic stretching vibration bands that correspond to (N-N=N) Azid band which are appeared, also stretching vibration bands that coincide to (NH) and (NH2) primary amine band which are disappeared. Figure. The FTIR spectra of compounds (6-10) have important characteristic stretching vibration bands that coincide to (C=O or C=S) cyclic amide band which are appeared ^1^H NMR spectra of prepared compound [6]

Singlet 1H of -NH (triazole ring) (8.33); multiplet 6H of aromatic rings (7.36- 7.75); Singlet 1H of –NH group (4.64); Singlet 1H of –CO-CH group (3.55); Singlet 1H of –CH-N group (3.30); Singlet 3H of -N-CH3 group (3.12); Singlet 2H of -CH2 group (3.07); Singlet 3H of –CH3 group (1.84).
Fig. 1: FTIR & $^1$HNMR spectra of compound (1)

Fig. 2: FTIR & $^1$HNMR spectra of compound (3)
Anti-cancer activity\(^{10-15}\)

One, two, three Triazoles have long been derived and have been mainly in a place in order to discover for their likelihood of occurring in Anti malignant (HepG2, MOLT-3)chemotherapy. Many groups have conducted focused research in this particular area, Odlo et al. Researcher discovered a new genetic link to the causes of the disease. A series of cis-restricted 1,5-disubstituted 1,2,3-triazole analogs of combretastatin.

A primary Anti malignant (HepG2, MOLT-3) Activity assay was accomplish a task on a panel of approximately 30 hamantum tumor cell lines derived from two neoplastic diseases (HepG2, MOLT-3) in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute (NCI). The tested compounds were supplementary to the culture at a single concentricity (10–5 M) and the cultures were incubated for 48 h. Endpoint determinations were made with a protein binding dDye.
Experimental:

Instrument sand apparatuses:

1- The infra-red spectra of the synthesized compoundsswere recorded using FTIR 8400 Fourier transform infrared spectrophotometer of SHIMA-DZU Company as a potassium bromide disc in the wavenumber wavenumber range of (4000-4000) cm⁻¹, University of Baghdad, College of Science, Department of Chemistry.

2- ¹H NMR and ¹³C NMR spectra were recorded on nuclear magnetic resonance Bruker spectrophotometer model Ultrasheild- 400- MHz using Tetramethylsilane internal standard and D₂O as solvent (Isfahan- University of Technology (IUT), Iran).

3- The Melting point was determined by the open capillary method using the hot stage- Gallenkamp-melting point

Ethical Clearance: A local Ethical Committee reviewed and approved the study.

Chemicals: -

The entire chemicals used in this work were of the highest purity available (98-99%) and they were used without further purification.

- Preparation of new ammine 5-(4-nitrophenyl)-1, 3, 4-thiadiazol-2-amine compound by Bharadwaj et al (2010) performed the condensation of (4-nitrophenyl)-1, 3, 4-thiadiazol-2-amine [1] under microwave oven. The structures of the synthesized compound were confirmed on the basis of spectral and elemental analysis. The synthesized compounds were found in better yield than in conventional methods and also screened for in vitro anticancer study.

- Preparation of 2-chloro-N-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)-acetamide. The literature procedure was used with some modifications. The 4-nitrophenyl)-1, 3, 4-thiadiazol-2-amine (0.02 Mole) was dissolved in DMF (20mL) and then cooled at (0-5°C) and 2-3 drops of TEA were added. Chloroacetyl chloride (0.02 Mole) in DMF (20 mL) was slowly added to R.B.F (Round bottom flask) with vigorous stirring for 3 hours at room temperature. The obtained product was filtered and washed with ether, recrystallized from suitable solvent.

- Preparation of N-5-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)thiazole-2,5-diamine or N-5-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)oxazole-2,5-diamine. The literature procedure was used with some modifications. In 100 mL R.B.F (0.02 Mole) of compound (1) and (0.02 Mole) of thiourea / urea was dissolved in 1,4-dioxane (20mL) and the mixture was refluxed for 18 hours. The obtained product was filtered and recrystallized from suitable solvent.

- Preparation of Azide by primary amine. The literature procedure was used with some modifications. In 100 mL R.B.F (0.02 Mole) of hetero primary amine compound and (0.02 Mole) of triethyl amine/CH₂Cl₂ was dissolved in methanol (20mL) and the mixture was refluxed for 18 hours. The obtained product was filtered and recrystallized from suitable solvent.

-- Preparation of 1,3,4-thiadiazol-2-yl-oxazol-5-aminedervative. To 20 mL of hot ethanol, (0.005 mol) of benzaldehyde and (0.0025 Mole) of compounds (16-21) were dissolved. To this mixture, 1.0 mL of glacial acetic acid was added. The reaction mixture was then refluxed in the water bath for 12 hours. Completion of the reaction was monitored by TLC (benzene with ethylacetate). The mixture was allowed to stand for 24 hours at room temperature. The product was collected and recrystallized with a suitable solvent.

Conclusion

The present study gives rise to the following conclusion, This work includes four parts: the first part Preparation of 5-(4-nitro phenyl)-1,3,4-thiadiazol-2-amine by the reaction of p-nitrobenzoic acid with thiosemicarbazide, the second part prepared 2-chloro-N-(5-(4-Nitrophenyl)-1,3,4-thiadiazol-2-yl) Acetamide by reaction Chloroacetyl chloride with compound and part three contain reaction compound(2) with urea/thiourea to get compounds cyclization by using urea (thiourea) finally step reaction these compounds with different aldehydes and ketones in glacial acetic acid present work is synthesis new derivatives of 1,2,3-Triazole having heterocyclic compounds on ring from the reaction of azine with a different reagent to give (1,2,3- Triazole) rings. The 1,2,3-Triazoles It has been derivatives A long time ago and has been Scout mainly for their potential in anti-Tumor chemotherapy the prepared of 1,2,3-Triazole financial products have good AAnti malignant (HepG2, MOLT-3) Activity.
Many groups have carried out focused research in this particular. The tested compounds were supplementary to the culture at a single concentricity (10−5 M) and the cultures were incubated for 48 h. Endpoint determinations were made with a protein binding dye.

**Source of Funding:** Nil

**Conflicts of Interest:** Nil

**Ethical Clearance:** A local Ethical Committee reviewed and approved the study.

**References**


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Forensic Human and Non-human Blood Discrimination using Mitochondrial 12S rRNA gene

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²Assistant Professor at Biology Department, College science-University of Babylon, Iraq

Abstract

Background: The blood samples of spots typing regards most important step in forensic analysis. Determining the blood affiliation as human on non-human is not enough as a forensic tool leading to the truth. The mitochondrial 12S rRNA gene are suitable for taxonomy and species identification, especially for the discrimination of even closely related species. The current study aimed to showed the validity of designed 12S rRNA species specific primer pairs for human and nonhuman animal blood typing as a forensic tool.

Methodology: Seventy two blood samples were collected from Homo sapiens, Ovis aries, Capra hircus and Bos taurus (18 blood samples from each). Five milliliter were withdrawn and placed in EDTA-tube and stored in refrigerator for further processing. Four sets of species specific primer pairs targeting mitochondrial 12S rRNA were designed and checked. PCR and sequencing were performed and sequences were analyzed and register in GenBank.

Results: the results revealed that, the amplicon of 12S rRNA gene of Homo sapiens were 800bp, Ovis aries were 560bp, Capra hircus were 460bp, and Bos taurus were 600bp. The first confirmatory test for validity and specificity of designed primer pairs is no amplification for intraspecies primer pairs (i.e. using homo12S rRNA primer pairs to amplify Ovis aries, Capra hircus, and Bos taurus 12S rRNA will not give product and vice versa). The second confirmatory test is the results of sequencing. The identity percentage and alignment of sequences results of amplified 12S rRNA gene of homo sapiens, Ovis aries, Capra hircus and Bos taurus revealed that, the similarity percentage ranged from 98.03% to 100%.

Conclusion: The current study conclude that, validity and accuracy of designed 12S rRNA species specific primer pairs for human and nonhuman animal blood typing as a forensic tool and there is no intraspecies cross amplification.

Keywords: Homo sapiens, Ovis aries, Capra hircus and Bos taurus, 12S rRNA, Sequencing

Introduction

The assignment of blood samples is a fundamental aspect of forensic investigations, mainly for vehement crimes. The important step in forensic that is often skipped or unnoticed is the determination that a bloodstain is of human or nonhuman origin at level of species[1]. The mitochondrial genome consists of multiple copies of 116,569 bp, double stranded mitochondrial DNA (mtDNA) molecules and located adjacent to the OXPHOS system in the matrix. It is haploid in countryside, and thus should evolve four times faster than the average nuclear gene. Hence, mtDNA can be used to track divergence in very closely related taxa and even within species[2-4]. Mitochondrial 12SrrRNA has proven to be a useful molecular marker for better conservation and management of the endangered species. Mitochondrial DNA present in high copy numbers, survive for prolonged periods and absence of recombination when compared with nuclear DNA making it suitable for forensic investigations [5,6]. The mitochondrial 12S rRNA gene are suitable for taxonomy and species identification, especially for the discrimination of even closely related species. It is widely used to differentiate between human from non-human species. Genotyping using 12S rRNA gene
provide cost and time effective results, and yielded information not only for species identification but also for individualization[7,8]. The current study aimed to showed the validity of designed 12S rRNA species specific primer pairs for human and nonhuman animal blood typing as a forensic tool.

Methodology

Sampling:

Seventy two blood samples were collected from Homo sapiens, Ovis aries, Capra hircus and Bos taurus (18 blood samples from each). Five milliliter were withdrawn and placed in EDTA-tube and stored in refrigerator for further processing.

Polymerase Chain Reaction:

G-spinTM Total DNA Extraction Kit (was used to extract mitochondrial DNA from blood of different species according to the manufacture’s protocol instructions (Intronbio/Korea). All extracted DNA samples were submitted for Nanodrop to ensure adequate purity and concentration required for PCR. Setting done by selection dsDNA measuring mode for 2 μl of sample. Blanking were performed using 2 μl microliter of elution buffer and then measuring the DNA samples concentration and purity[9].

The primer pairs were designed using the following reference sequences: Homo sapiens (NC_012920.1, ), Ovis aries: (NC_001941.1), Capra hircus (NC_005044.2) and Bos taurus (NC_006853.1). Primer 3 software was used to design the specific primer pairs: Homo12S-F: CATCCCGTTCCAGTGAGTT, Homo12SR: TACTTGAGGAGGGTGACGGG (800bp); Ovis 12S-F: GCCTGTGATAGCTGGTTGT, Ovis 12S-R: ACGTTTAGTCACTGGGACGG (560bp); Capra 12S-F: GGTGAGTAAACGCCCTCCAA, Capra 12S-R: ATAAAGCACCAGCAGCACCT (460bp); Bos 12S-F: TAAATCTCGTCCAGCCACCC, Bos 12S-R: TGGGCTGCTCAGCCCTAAAT (600bp). The specificity of designed primer pairs were checked using online NCBI/primer blast[10,11]. The PCR conditions were calculated using online Protocol Optimize writer software. The conditions were illustrated in table (1).

Sequence Analysis and Submission

Trimming of sequences were perform by FinchTV and then submitted to NCBI-BLASTN to see the identity of sequences with reference sequences within NCBI data base. All trimmed and confirmed sequences then submitted for GenBank to register all sequences within the database. The gathered accession no. after submission verified the assignment of the sequences samples to the intended species.

Table (1): PCR Conditions

<table>
<thead>
<tr>
<th>Primer</th>
<th>Conditions</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Homo 12S-F, Homo 12S-R</td>
<td>1 95°C, 30sec. 60.3°C, 72°C 1 72°C</td>
<td>This study</td>
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<tr>
<td>Ovis 12S-F, Ovis 12S-R</td>
<td>1 95°C, 30sec. 59.3°C, 72°C 1 72°C</td>
<td>This study</td>
</tr>
</tbody>
</table>
Results and Discussion

The results of PCR reveal that, the amplicon of 12S rRNA gene of Homo sapiens were 800bp, *Ovis aries* were 560bp, *Capra hircus* were 460bp, and *Bos taurus* were 600bp (Figure 1: A,B,C,D respectively). The mitochondrial genes have been used as molecular markers to identify mammals, birds, shrimp, and other
species using species-specific primers that amplify the 12S rRNA or 16S rRNA gene regions from mtDNA\cite{12,13}. Gene loci on the mitochondrial genome have been used in species identification due to the greater sequence variation at this non-coding locus, it is now being used as a tool for identifying the presence of particular species within mixture of many species\cite{14,15}.

**Figure (1):** 1.5% Agarose gel electrophoresis for: A: (800bp) amplicon of Homo sapiens 12S rRNA gene. lane H1-H18 represent samples. B: (560bp) amplicon of Ovis aries 12S rRNA gene. lane O1-O18 represent samples. C: (460bp) amplicon of Capra hircus 12S rRNA gene. lane C1-C18 represent samples. D: (600bp) amplicon of Bos taurus 12S rRNA gene. lane B1-B18 represent samples. M represent 100bp DNA ladder.

The first confirmatory test for validity and specificity of designed primer pairs is no amplification for intraspecies primer pairs (i.e. using homo12S rRNA primer pairs to amplify *Ovis aries*, *Capra hircus*, and *Bos taurus* 12S rRNA will not give product and vice versa). The second confirmatory test is the results of sequencing. The identity percentage and alignment of sequences results of amplified 12S rRNA gene of *homo sapiens*, *Ovis aries*, *Capra hircus* and *Bos taurus* with database were illustrated in tables (2,3,4,5) respectively. The PCR amplification and sequencing of mitochondrial DNA 12S rRNA gene could be used for unambiguous species identification of mammalian. The technique has the advantage that only a small amount of material is required. The method could be used even for biological material stored at ambient temperature for prolonged periods of time\cite{16}. Mitochondrial gene12S rRNA the specificity of the primers was tested against 29 animal species including mammals, birds, and fish, as well as 8 plant species. Analysis of experimental feedstuffs demonstrated the detection of each target species in the range of 0.1 to 100\%\cite{17}. Frezza et al., (2003)\cite{18} Polymerase chain reaction allows direct species identification, but high temperatures involved in rendering animal products (133°C for 20 min) cause fragmentation of DNA. Amplifying and sequencing a fragment of mitochondrial DNA that codes for 12S ribosomal RNA, to identify the species origin of non-human casework samples thus mtDNA results are obtained and is especially useful degraded samples\cite{19}.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Sequence ID</th>
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<tr>
<td>IHS12-1</td>
<td>MH444415.1</td>
<td>100.00%</td>
<td>731</td>
</tr>
<tr>
<td>IHS12-2</td>
<td>MK069579.1</td>
<td>99.86%</td>
<td>771</td>
</tr>
<tr>
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<td>MK069579.1</td>
<td>100.00%</td>
<td>697</td>
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<td>654</td>
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<td>100.00%</td>
<td>701</td>
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<td>IHS12-8</td>
<td>MK069579.1</td>
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<td>653</td>
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</table>

Table (2): Identity of blasted isolates (IHS12-1 to IHS12-8) with reference sequences of highest identity percentage
Table (3): Identity of blasted isolates (IOA12-1 to IOA12-8) with reference sequences of highest identity percentage

<table>
<thead>
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<tr>
<td>IOA12-6</td>
<td>MG489885.1</td>
<td>100.00%</td>
<td>283</td>
</tr>
<tr>
<td>IOA12-7</td>
<td>MG489885.1</td>
<td>99.38%</td>
<td>486</td>
</tr>
<tr>
<td>IOA12-8</td>
<td>KU681201.1</td>
<td>98.64%</td>
<td>367</td>
</tr>
</tbody>
</table>

Table (4): Identity of blasted isolates (ICH12-1 to ICH12-8) with reference sequences of highest identity percentage

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Sequence ID</th>
<th>Identities</th>
<th>No. of Aligned Base</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICH12-1</td>
<td>LR025741.1</td>
<td>100.00%</td>
<td>408</td>
</tr>
<tr>
<td>ICH12-2</td>
<td>LR025741.1</td>
<td>99.75%</td>
<td>408</td>
</tr>
<tr>
<td>ICH12-3</td>
<td>LS992659.1</td>
<td>100.00%</td>
<td>408</td>
</tr>
<tr>
<td>ICH12-4</td>
<td>LR025741.1</td>
<td>99.75%</td>
<td>408</td>
</tr>
<tr>
<td>ICH12-5</td>
<td>LS992662.1</td>
<td>99.75%</td>
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</tr>
<tr>
<td>ICH12-6</td>
<td>LR025741.1</td>
<td>100.00%</td>
<td>406</td>
</tr>
<tr>
<td>ICH12-7</td>
<td>LR025741.1</td>
<td>99.75%</td>
<td>408</td>
</tr>
<tr>
<td>ICH12-8</td>
<td>HQ996553.1</td>
<td>100.00%</td>
<td>324</td>
</tr>
</tbody>
</table>
Table (5): Identity of blasted isolates (IBT12-1 to IBT12-8) with reference sequences of highest identity percentage

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Sequence ID</th>
<th>Identities</th>
<th>No. of Aligned Base</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBT12-1</td>
<td>EU177870.1</td>
<td>100.00%</td>
<td>544</td>
</tr>
<tr>
<td>IBT12-2</td>
<td>MK028750.1</td>
<td>100.00%</td>
<td>537</td>
</tr>
<tr>
<td>IBT12-3</td>
<td>EU177870.1</td>
<td>100.00%</td>
<td>545</td>
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<td>100.00%</td>
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<tr>
<td>IBT12-5</td>
<td>MK028750.1</td>
<td>100.00%</td>
<td>543</td>
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<tr>
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<td>IBT12-8</td>
<td>EU177869.1</td>
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</tbody>
</table>

Sensitivity, specificity and accuracy of designed primer pairs can be achieved using following equation depending upon sequencing as gold standard [20]. (Table 6).

Table (6): Sensitivity, specificity and accuracy of designed primer pairs

<table>
<thead>
<tr>
<th>Test</th>
<th>PCR of 12S rRNA gene</th>
<th>Homo sapiens</th>
<th>Ovis aries</th>
<th>Capra hircus</th>
<th>Bos taurus</th>
</tr>
</thead>
<tbody>
<tr>
<td>True Positive</td>
<td></td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>False Positive</td>
<td></td>
<td>0</td>
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<td>0</td>
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</tr>
<tr>
<td>True Negative</td>
<td></td>
<td>0</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>False Negative</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>1(100%)</td>
<td>1(100%)</td>
<td>1(100%)</td>
<td>1(100%)</td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td>1(100%)</td>
<td>1(100%)</td>
<td>1(100%)</td>
<td>1(100%)</td>
<td></td>
</tr>
<tr>
<td>Accuracy</td>
<td>1(100%)</td>
<td>1(100%)</td>
<td>1(100%)</td>
<td>1(100%)</td>
<td></td>
</tr>
</tbody>
</table>

The results showed no cross-reactivity of designed primer pairs and the PCR assay based on the designed primer pairs will be simple, fast, sensitive, specific, and cost-effective.

Registration of Sequences in GenBank:

All the 32 sequence of 12S rRNA gene were submitted to GenBank for registration. After checking and revision the following accession numbers were donated:

**12S rRNA Homo sapiens (Human):** MN203699, MN203700, MN203701, MN203702, MN203703, MN203704, MN203705, MN203706.

**12S rRNA Ovis aries (Sheep):** MN202782, MN202783, MN202784, MN202785, MN202786, MN202787, MN202788, MN202789.

**12S rRNA Capra hircus (Goat):** MN197657, MN197658, MN197659, MN197660, MN197661, MN197662, MN197663, MN197664.
12S rRNA *Bos taurus* (Cow): MN197785, MN197786, MN197787, MN197788, MN197789, MN197790, MN197791, MN197792.

**Conclusion**

The current study conclude that, validity and accuracy of designed 12S rRNA species specific primer pairs for human and nonhuman animal blood typing as a forensic tool and there is no intraspecies cross amplification.

**Ethical Clearance:** The project plan displayed on the scientific committee and scientific ethical committee of the department of Biology-college of science at university of Babylon and get approval

**Source of Funding:** There is no funding source and it is completely covered by authors

**Conflict of Interest:** There is no conflict of interest

**References**


Antifungal Activity of Crude and Phenolic Extract to Rice Crusts and Chemical Pesticide (Blitinute) in Inhibition of Fungi Isolate from Rice Seeds

Angham Najah Al-khafaji1 Athraa Harjan Muhsin2, Mays Talip Abdallah3

1Al-Furat Al-Awsat Technical University, Kufa Technical Institute, 2 University of Kufa, Faculty of Agriculture, 3University of Al-Naharin, Faculty of Biotechnology

Abstract

Objective: The major goal regarding the presented study is to evaluate the impact of the Rice extract from Rice Crusts and chemical pesticide (Blitinute) on the growth of pathogenic fungi.

Method: Rice seed samples were collected directly from farmers field for isolating significant pathogenic fungi related to the seeds. Anti-fungal activity assay of the crude, Phenolic extract and chemical pesticide were determined by agar plating-method against some pathogenic fungi.

Result: obtained results showed the effect concentration of rice seed extract on growth of fungi especially phenolic extract had wide spectrum antifungal activity than crude extract. when the use of chemical pesticide (Blitinute) at a concentration of 0.3% with reaching rate of inhibition to 100% to Fusarium graminerum , Penicillium viridicatum, while (88.8) the same concentration for the Aspergillus fumigates. In another than the use Integrations between two extract and chemical pesticide (Blitinute) to high effect against test fungi than one extract and chemical pesticide.

Conclusion: This study demonstrates that the presence of three pathogenic fungi (Aspergillus fumigatus , Fusarium graminerium, Penicillium viridicatum) related to the rice grains have been controlled at various concentrations of two extract (crude & phenolic) and chemical pesticide totally inhibited radial growth regarding all test fungi.

Key Words: Rice Crust, Crude & phenolic extract, Chemical pesticide, pathogenic fungi.

Introduction

Continuously plants have been endangered through various pathogenic micro-organisms existing in their environments. Globally, phytopathogenic fungi is specified the major beneficent pest with regard to the farming harvests 1. A Considerable amount of the agricultural products globally and in the country are not suitable for human’s consumption, also they are not healthy because grains are polluted with mycotoxins, created via Penicillium, Fusarium, and Aspergillus. The reports have indicated that over three-hundred 300 fungal metabolites have high toxicity towards humans as well as animals, and 25% of the cereals in the world have been contaminated with certain mycotoxins 2. The diseases produced from fungi form cause a significant loss of many economic crops in the world. The largest effect of fungi with regard to the decrease in efficiency of crops or post-harvest losses and leads to a huge loss to mankind 3.

Rice can be specified as a food of high significance that result in approximately 35% of the industrial costs, approximately 70% of the average calorie consumptions in addition to 93% of total produced food 4. Worldwide, Rice (Oryza sativa) is a major cereal harvest. With regard to India, rice occupy the first in area of approximately 42. 24 million hectares and produce approximately 82 million tons 5. Many studies on rice grain spotting as well as on its control were achieved home and abroad, yet the statistics regarding storages rice grain contain mycoflora as well as its control are not enough 6.
Plants are producing many types secondary metabolites regarding the low-molecular-mass which are typically isn’t needed for basic metabolic procedures related to plants. A lot of secondary metabolites regarding these plants are recognized as allelochemicals which enhanced the protection against other plant competitions, insect/animal predation or microbial adhesion. Allelochemicals might be of high importance in the biological control regarding phytopathogens and weeds. A lot of plant products and plants have antimicrobials against plant pathogenic fungi. Control seed-borne fungal diseases and inhibiting the biodeterioration related to the grains with using use seed treatment is cost-effective and harmless approach. Reports indicates that extracts of many plants were showing antibacterial, anti-fungal as well as insecticidal properties under laboratory trails. Appear plant metabolites in addition to the plant-based pesticides are considered as more effective alternatives since they have minimum environmental impact and dangers to the humans in a way similar to the synthetic pesticides. Earlier, a lot of plant extracts which have source of bio-pesticide because contain substances inhibit the growth of plant pathogens and reduced the risk to environment and health of human. The presence of antifungal compounds in higher plants has long been recognized as important agents for the control of certain plant diseases. Recently, many researchers in the world show interest in the application of plant product as bio-pesticide.

**Aim of Objective:-**

The present work aim was to evaluate the effect of Rice extract from Rice Crusts and chemical pesticide (Blitinute) on the growth of pathogenic fungi.

**Methodology**

**Isolation of fungi from rice seeds:**

Isolate the main pathogenic fungi from seed was collected rice seed samples directly from farmer’s field. During the seventh day of incubation, examine the mycoflora seed samples with use microscopes. Identification 6 fungi based on growth characteristics, spore mycelial morphology, in addition to other features with applying standard guides.

The three species of *Aspergillus* and one species of each *Fusarium graminum*, *Penicillium viridicatum*, *Cladosporium* spp were subculture using Potato Dextrose Agar (PDA) medium.

**Preparation of Rice Seed Extracts:**

- **Crude Extract**

  Weight 100g of Crusts rice powder and put in a 1000 ml glass flask and add 200 ml of ethyl alcohol and leave for 48 hours, taking into reflection the shaker. Periodically from time to time, the filter was filtered using filter paper (Whatman No.4) and by vacuum. The extract was concentrated using (Rotary vacuum Evaporator) at a temperature of 45ºC, then the weight of the concentrated extract, the purpose of the biological activity testing to the raw extract in the test fungi, take 0.3g of dry extract and dissolve in 1ml of ethyl alcohol 99% and then complete the volume to 10ml by distilled water. Concentration is 3%. For control, it is 1ml of ethyl alcohol and full size with distilled water to 10 ml.

- **Phenolic Extract**

  The method was followed by to extract the phenolic compounds from the Crusts rice, take 20g dry weight of the crusted crust and put in a 500ml glass flask, and add 400 ml of 2% HCl. The phenolic compounds were extracted using a water bath at 100 °C for one hour. After the end extraction process, the solution was cooled and filtered with the filter paper type Whattman No.1 and put filter in the separating funnel and added an equal volume of n-propanol, and then added to the amount from NaCl to reach the state of saturation, then formed two layers, isolated the upper layer containing phenolic compounds, and focused on rotary evaporator, then placed in the oven at 40°C to dry, and reduced in a refrigerator for use, For the purpose of testing the biological activity of the phenolic extract in the test fungi, take 6g of phenolic extract and dissolve in 8ml of ethyl alcohol 90% and then complete the volume to 25ml by distilled water. Concentration is 24%. For control, it is 8ml of ethyl alcohol and full size with distilled water to 25 ml.

**Anti-fungal activity assay:**

The preparation of media PDA in two flask the volume each flask about 500ml with the 400 media/flask, then sterilization with autoclave, after sterilization leave to cool, before solidification of the medium to content pour 18 plate, in average 20ml/media, 9 plate content extract to 1ml each plate, while another 9 plate content solution ethyl alcohol and distilled water (control treatment) at 1ml/plate, after solidification of the medium to culture 5mm disc of 7-day-old culture of
test fungi have been inoculated, there are 3 replicates have been maintained for all extracts. Plates have been incubated at a temperature of 22±1 Celsius for 7 days. after the percentage inhibition regarding mycelia growth has been estimated with the use of formula based on 14.

\[
\% \text{ inhibition} = \frac{dc - dt}{dc} \times 100
\]

where:

dc = average increase in mycelia growth in control.
dt = average increase in mycelia growth in plate content extraction.

Result

Isolation and Identification:

Show fungi on seed borne the percent incidence associated with rice seed, the data revealed that the *Aspergillus fumigates*, *Fusarium graminerum*, *Penicillium viridicatum* with the high frequencies, the percent of frequencies include (93,90,89)% respectively. While other include *Aspergillus terrens*, *Aspergillus niger*, *Cladosporium* spp the percent (77, 63, 58)% respectively .

The results of Reagent use to detect crude extract of rice crust, showed that the alkaloids reagent includes (Drakandroff) negative, while positive of (Mayer, Tannic). Phenolic Reagent include (Lead acetate) positive, while negative of Potassium hydroxide. Turpinnes Reagent include (Foam & Mercury chloride) negative.

Effect concentration of rice seed extract and chemical pesticide (Blitinate) in growth inhibition test fungi:

Figure (1) indicates that decrease colony diameters by gradient concentration, with a 5% concentration being the most effective among concentrations (46.55, 52.22, 51.33) for fungi *Aspergillus fumigates*, *Fusarium graminerum*, *Penicillium viridicatum* respectively, compared to control treatments.

![Figure (1): Effect crude extract of Rice Seed to different concentrations (0,1,3,5)% on growth inhibition of test fungi](image1)

![Figure (2): Effect phenolic extract of Rice Seed to different concentrations (0,1,3)% on growth inhibition of test fungi](image2)
Figure (2) show that the phenolic extract of rice seed give high efficiency at 3% concentration as decrease the growth rates of the studied fungi to (45.55, 51.11, 41.11) for fungi Aspergillus fumigates, Fusarium gramineum, Penicillium viridicatum respectively.

The results are shown in figure (3) to decrease the growth rates of fungi and especially Fusarium gramineum, Penicillium viridicatum when the use of chemical pesticide (Blitinute) at a concentration of 0.3% with reaching rate of inhibition to 100% while (88.8) the same concentration for the Aspergillus fumigates.

![Graph showing inhibition zones for different concentrations and fungi](image)

Figure (3): Effect chemical pesticide (Blitinute) to different concentrations (0,0.1,0.3)% on growth inhibition of test fungi

Integration activity between Rice crusts extract and chemical pesticide (Blitinute) in inhibition of test fungi

The result in table (1) shown that the use integrations between crude extract at concentration 5% and chemical pesticide at concentration 0.3 to reduce contamination of seed by fungi Aspergillus fumigatus, Fusarium gramineum, Penicillium viridicatum to (89, 94, 80) respectively.

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Active Concentration</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus fumigatus</td>
<td>5% crude extract + 0.3 chemical pesticide (Blitinute)</td>
<td>89</td>
</tr>
<tr>
<td>Fusarium gramineum</td>
<td>5% crude extract + 0.3 chemical pesticide (Blitinute)</td>
<td>94</td>
</tr>
<tr>
<td>Penicillium viridicatum</td>
<td>5% crude extract + 0.3 chemical pesticide (Blitinute)</td>
<td>80</td>
</tr>
</tbody>
</table>

While use integrations between phenolic extract at concentration 3% and chemical pesticide at concentration 0.3 to reduce contamination of seed with the same fungi to (87, 99, 81) respectively, as show in table (2).

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Active Concentration</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus fumigatus</td>
<td>3% phenolic extract + 0.3 chemical pesticide (Blitinute)</td>
<td>87</td>
</tr>
<tr>
<td>Fusarium gramineum</td>
<td>3% phenolic extract + 0.3 chemical pesticide (Blitinute)</td>
<td>99</td>
</tr>
<tr>
<td>Penicillium viridicatum</td>
<td>3% phenolic extract + 0.3 chemical pesticide (Blitinute)</td>
<td>81</td>
</tr>
</tbody>
</table>
The result in table (3): show efficiency use two extract (crude & phenolic) at concentration 5% & 3% respectively and chemical pesticide in concentration 0.3 to reduce seed contamination with fungi *Aspergillus fumigates*, *Fusarium gramineum*, *Penicillium viridicatum* to (94, 100, 90) respectively.

**Table (3): Integrations use between active concentration of crude, phenolic extract and chemical pesticide (Blitinute) against test fungi**

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Active Concentration</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus fumigatus</em></td>
<td>0.3 chemical pesticide (Blitinute) + 5% crude extract + 3% phenolic extract</td>
<td>94</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Fusarium gramineum</em></td>
<td>0.3 chemical pesticide (Blitinute) + 5% crude extract + 3% phenolic extract</td>
<td>100</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Penicillium viridicatum</em></td>
<td>0.3 chemical pesticide (Blitinute) + 5% crude extract + 3% phenolic extract</td>
<td>90</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Discussion**

Botanist can be specified as rich storehouse related to the natural chemicals which might be exploited to be used as pesticides. Reports indicates the overall quantity regarding plant chemicals might be over 4,000,000 and of these 10,000 are to be create secondary metabolites which are of high importance in the plant’s protective them. Previous researches indicate that anti-microbial activity includes anti-fungal properties with the use of diverse classes regarding the extracts. The reports indicated creating considerable decrease in nutritious quality and seed quality because of the association to fungi ranges. Other studies have proven the effective role of medicinal plants in eliminating various pathogen. Not been described a lot of species regarding advanced plants have extremely less studied for chemical or biologically active components and novel sources of commercially valuable pesticides. Various studies indicate a major alternative for better alternatives plant metabolites and plant based pesticides as recognized to have reduced environmental impact and hazard to the consumers when compared to the synthetic pesticides. This study is consistent with other studies in the inhibition of pathogen using probiotics in the treatment of multiple microorganism resistant to antibiotics.

The attributed to destruction regarding the occurrence of seed borne fungi which might have killed embryo of seeds because of the ability of extracts for increasing seed germination as well as seedling development. Such results are in accordance with the results of who indicated that leaf extracts of *C. viscosum* have increased the growth of seeds as well as improving the development regarding rice seeds. Plant extracts are considered to be bio-pesticide control agent which are of high importance due to 2 certain reasons. The first on is it can be safely used for individuals as well as environmental accumulation. The second reason is its capability for controlling pathogens as well as inhibiting pathogens from the emerging resistance to fungicide. The main step is application of the bio-pesticides for using natural products from the plants as measure for controlling and inhibiting plant diseases. This result consistent with another studies. Such results are in accordance with for showing the impacts of 2 biocides (“Bacillin and Floramyle”) on inhibition the fungus growth (*Aspergillus flavus* and *Aspergillus niger*) for rice seed contamination.

**Conclusion**

The main step of this study demonstrates in the
development of pesticides based on plant that are environmentally approachable for the controlling fungi on seed-borne and the improvement of commercial preparations of plants, and revealed the presence of three pathogenic fungi (*Aspergillus fumigatus*, *Fusarium graminerium*, *Penicillium viridicatum*) on rice grains were controlled in different concentrations, the two extracts (crude and phenolic) and chemical pesticides completely inhibit the radial growth of all test fungi.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** The authors declare no conflict of interest.

**Ethical Clearance:** All experimental protocols were approved under the Al-Furat Al-Awsat Technical University, Kufa Technical Institute/Iraq and all experiments were carried out in accordance with approved guidelines.

**References**

19. Harjan.A and Al-Khafaji, A.N. Study the effect of some plant extracts efficiency to reduce the pathogenesis *Candida albicans* isolates from


Identification of Farnesoid X Receptor as a Novel Nuclear Receptor Sensing for Gallstone Diseases

Antesar Rheem Obead1, Maha Fahdil Mohammed2, Mohend AL. Shalah3

Babylon University, College Of Medicine, Biochemistry Dept., Hilla, Iraq

Abstract

Objective:- The bile acid-make active nuclear receptor farnesoid X receptor (FXR) acting an significant part in lipid then glucose metabolism, besides furthermore, it controls numerous lipid carriers complicated in stone disposition. We observed whether a useful single nucleotide polymorphism (SNP) in FXR (82 G>T) predisposed the gallstone development.

Material and Method: - The polymorphism were confirmed by polymerase chain reaction monitored by PCR amplicons were commercially sequenced from both (forward and reverse) termini according to education manuals of the sequencing company (Macrogen Inc. Geumchen, Seoul, South Korea), in two group match by age ≥20 : patient with gallstone (n=140) and stone-free control (n=140).

Result: - For Sequencing of the 195 bp region within the NR1H4 (FXR) gene Within this locus, twenty samples were included in the present study that had shown to amplify the NR1H4 (or FXR) genetic sequences in the chromosome number 12. The latter gene is responsible for encoding on nuclear receptor subfamily 1 group H member 4 (NR1H4). The alignment results of the 195 bp samples revealed the presence of one SNP occurred in this position in sample no. 10 within the analyzed twenty samples in comparison with the referring reference DNA sequences. The sequencing chromatogram of the observed substitution SNP, as well as its detailed annotations, were documented, and the chromatogram details of the observed SNP were shown according to their positions in the PCR amplicon, in which samples no. 10-20 had shown this (T82G) variation.

Conclusion: - the education presented that the variation allele of the communal FXR 82G>T polymorphism was significantly associated with stone formation in Iraq patients. The association is possibly concluded the effect of the FXR 82G>T polymorphism on the expression of the efflux carriers for lipid trails in hepatic.

Key word: - FXR, PCR, Gallstone, polymorphism, sequencing analyzing.

Introduction

Gallstones are aggregation of hard substance that forms inside gallbladder1, when there is disequilibrium in the constitution of bile such as more cholesterol, accumulated amount of pigment material and/or decreased amount of bile acid2. Gallstones may also result from dysfunction of gallbladder contraction3. Nuclear receptors (NRs) are ligand-activated transcription factors with important roles in different aspects of human physiology and development of gallstone4. FXR regulates the expression of various genes involved in bile acid, lipid and glucose metabolism, by binding to DNA either as a monomer or an heterodimer with a common partner for NRs, Retinoid X Receptor (RXR). FXR is highly expressed in the liver, intestine, kidney and adrenals5. Two known FXR genes exist, the Fxrα and Fxrβ. Fxrα gene in humans encodes four FXRα isoforms (FXRα1, FXRα2, FXRα3 and FXRα4) as a result of the use of different promoters and alternative RNA splicing. Given the importance of the FXR in bile acid and in regulating the expression of the gene that are involved in gallstone formation, we examined the association between the functional SNP FXR 82G>T and gallstone disorder.

Material and Method

Object in this analysis include two groups matched by age ≥20 years, consecutive symptomatic patients (n...
with gallstone disease (GD), and healthy stone-free control subjects \( n = 140 \) confirmed by abdominal ultrasonography.

**DNA extraction and genotyping**

DNA was isolated from whole blood by Wizard® Genomic DNA Purification Kit Staining. FXR genotyping was performed as previously described \(^8\). Briefly, PCR was carried out in a reaction mixture of 25 μL containing 0.5 units of Taq DNA polymerase and 5 μL of template DNA with a concentration of 50 to 150 ng/μL, using primers 5’-GAGCCAGTGAACAGAAACCC-3’ (sense) and 5’GTGAGAGAGGACAGAGGTTG-3’ (antisense) \(^7\). Amplification reaction consisted of 30 cycles, with Initial Denaturation 95°C for 3 minute , annealing 61°C for 30 second, Extension 72°C for 1 minute , to yield 194bp of product FXR.

**Detection of farsenoid X Receptor (FXR) gene polymorphism (82 T>G)**

**PCR Product using Promega Master Mix**

PCR Product using Promega master mix was given sharp and apparent 195 bp band as figure (1) shown , therefore the study choose it in the rest of the work because of its high reliability

<table>
<thead>
<tr>
<th>M</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
</tr>
</thead>
</table>

![Figure (1): PCR Product using promega master mix on 1.5% agarose, 70V, and for 45 minute (5 µl of DNA loaded in each well), lane M: 100 bp ladder Lane 1-13 : PCR product.](image)

**Sequncing Method**

**DNA Sequencing of PCR amplicons**

The resolved PCR amplicons were commercially sequenced from both (forward and reverse) termini according to instruction manuals of the sequencing company (Macrogen Inc. Geumchen, Seoul, South Korea). Only clear chromatographs obtained from ABI sequence files were further analyzed, ensuring that the annotation and variations are not because of PC or sequencing artifacts. By comparing the observed DNA sequences of local bacterial samples with the retrieved neighboring DNA sequences of the NCBI Blastn engine, the virtual positions and other details of the retrieved PCR fragments were identified.

**Checking the novelty of SNPs**

The observed SNP was submitted to the dbSNP database to check their originality. Each particular SNP was re-positioned according to its place in the reference genome Subsequently, the determination of the presence of previous SNP was performed by viewing its corresponding dbSNP position. Then, the dbSNPs position for the detected SNP was documented.
Sequencing Result

1. Sequencing of the 195 bp region within the NR1H4 (FXR) gene

Within this locus, twenty samples were included in the present study that had shown to amplify the NR1H4 (or FXR) genetic sequences in the chromosome number 12. The latter gene is responsible for encoding on nuclear receptor subfamily 1 group H member 4 (NR1H4). This protein functions as a receptor for bile acids, and when bound to bile acids, binds to DNA and regulates the expression of genes involved in bile acid synthesis and transport (https://www.ncbi.nlm.nih.gov/gene/9971). The sequencing reactions indicated that the exact identity after performing NCBI blastn for these PCR amplicons (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch). Concerning the supposed 195 bp amplicons, NCBI BLASTn engine shown about 99% sequences similarity between the sequenced samples and the intended reference target sequences. By comparing the observed DNA sequences of these local samples with the retrieved DNA sequences (GenBank acc. NG_029843.1), the approximate positions and other details of the retrieved PCR fragments were identified (Fig. 2).

![Image of chromosome 12](https://www.ncbi.nlm.nih.gov/gene/9971)

Fig. 2. The exact position of the retrieved 195 bp amplicon that partially covered a portion of the FXR gene within chromosome 12 (GenBank acc no. NG_029843.1). The green arrow refers to the starting point of this amplicon while the blue arrow refers to its end point.

After positioning the 195 bp amplicons’ sequences within the FXR gene, the details of its sequences were highlighted, in terms of the positioning of both forward and reverse primers of the 195 bp amplified amplicon (Table 1).

<table>
<thead>
<tr>
<th>Amplicon</th>
<th>Referring locus sequences (5′ - 3′)</th>
<th>length</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA sequences within the FXR gene</td>
<td><em>GAGCCAGTGAACAGAAACCCACCCTCTTAAAGTTCTAAACTGGAAAAGTACTCC CCCAAAATGTTATCTAAGAGACTGTGTTTCCAGCTTACATGGAATTTGGAATTAAAG AACTTTTCTCTAAACATTTACAAATATCTCGATCGTAATGAAAGTTAACGTTAAA CCACACAACCTCTGCTCCTCTCCTCCTC</em></td>
<td>195 bp</td>
</tr>
</tbody>
</table>

* refers to the forward primer sequences

** refers to the reverse primer sequences

The alignment results of the 195 bp samples revealed the presence of one SNP occurred in this position in sample no. 10-20 within the analyzed twenty samples in comparison with the referring reference DNA sequences (Fig. 3).
Fig. 3. DNA sequences alignment of twenty local samples with their corresponding reference sequences of the 195 bp amplicons of the \textit{FXR} genetic DNA sequences. Each substitution mutation was highlighted according to its position in the PCR products. The symbol “ref” refers to the NCBI referring sequence, “S1-S20” refer to the samples 1 to 20, respectively.

The sequencing chromatogram of the observed substitution SNP, as well as its detailed annotations, were documented, and the chromatogram details of the observed SNP were shown according to their positions in the PCR amplicon, in which only sample no. 10 - 20 (S10-S20) had shown this T82G variation (Fig. 4).

Fig. 4. The pattern of the observed substitution mutation within the DNA chromatogram of the targeted 195 bp amplicons within the \textit{FXR} gene. The observed substitution mutations are highlighted according to their positions in the PCR products. The symbol “\textgreater{}\textless{}” refers to substitution mutation.
SNPs characteristics check

To elucidate the positions of the observed SNP with regard to their deposited SNP database of the sequenced 195 bp fragment, the corresponding position of the FXR gene was retrieved from the dbSNP server (https://www.ncbi.nlm.nih.gov/projects/SNP/). To find out the nature of the observed SNP, a graphical representation was performed concerning the FXR dbSNP database within chromosome 12 (GenBank Acc. No. NG_029843.1). By reviewing the dbSNP engine, it was found that this SNP was novel (Fig.5). However, this SNP was found to be located in an intronic position.

![Fig.5. The SNP’s novelty checking of FXR genetic single nucleotides polymorphism using dbSNP server. The identified SNP is marked with a green color.](image_url)

Therefore, it was found that this observed variant was not previously known and positioned in the intronic region within the FXR gene. To summarize all the results obtained from the sequenced 195 bp fragments, the exact position of the observed variation was described in the NCBI reference sequences. The pattern of the observed SNP in the 195 bp amplicons in comparison with the NCBI referring sequences of the FXR gene within the chromosome 12 (GenBank acc. no. NG_029843.1).

Discussion

It has been recommended that variations in monitoring protein coding genes, such as FXR, may possibly donate to the general dissimilarity for gene expression in tissues then thus effect stone nature then replies 8,9. This education is the first to report that the communal polymorphism (82 G>T) in FXR resulting in significantly condensed role of the gene was related with development of stone in Iraq patients through gallstone syndrome then that this suggestion remained important after regulating for the potential features.

To our information, there is no education reporting whether the gene for ABCG2, the main efflux carrier of , is a board of FXR, but then again unintended indication proposes that hepatic ABCG2 expression is controlled by some nuclear receptors, such as constitutive androstane receptor (CAR) then pregnane X receptor (PXR) 10, and the last is a board of FXR11. Consequently, it is likely that the condensed FXR action because of the 82G>T mutation may possibly decrease the ABCG2 expression by sound effects on PXR. It seems that the relationship between the FXR 82G>T polymorphism and stone development is additional apparent in issues homozygous for the wild-type alleles than in those with one or two duplicates of the variation allele of the FXR82G>T polymorphism. This consequence may possibly propose that the outcome of the FXR polymorphism on the gallstone is facilitated concluded a monitoring outcome on gene expression.

**Ethical Clearance:** Approval by scientific committee of Babylon Medical College (University of Babylon, Iraq) and the Biochemistry Department in the medical college.

**Source of Funding :** The funding body written out in full by self

**Conflict of Interest :** If any then mention it otherwise write it as nil

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Comparision between Nonvisualized Finding of 3D-Tof Mrv Cerebral and Cerebral Angiography Result

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1Department of Radiology, Faculty of Medicine, Universitas Airlangga, Surabaya (60131), Indonesia

Abstract

Background: Magnetic Resonance Venography (MRV) is a non-invasive method that evaluates the venous system without exposure to the radiation in the procedure compared to cerebral angiography and CT Venography. Nowadays, the use of MRV cerebral started to increase even though there are still many non-visualized (not visualized vein image) on MRV led to divergent interpretations or noncompliance to the findings of cerebral angiography, which is the gold standard to evaluate venous system. There has been few research comparing MRV findings with cerebral angiography so far.

Objective: The aim of this study is to evaluate the findings of non-visualized on MRV compare to cerebral angiography as the gold standard in helping the interpretation of examinations to avoid misdiagnosis.

Methods: Observational retrospective analytic study was used in this study that conducted at Husada Utama Surabaya Hospital from October 2013 to February 2014. 39 out of 49 patients selected as samples for fulfilling the inclusion criteria. The observation on the results of MRV and cerebral angiography procedures performed by two specialists of radiology, then the data were analyzed using SPSS.

Results: Based on the findings of abnormalities location from 39 samples, we found 35.45% abnormalities in the transverse sinus, 32.91% in the sigmoid sinus, 27.84% in the jugular vein, 2.53% in the straight sinus, and 1.27% in the superior sagittal sinus. The results of comparison between MRV test and cerebral angiography showed compliance at 21% hypoplasia with 88.9% of thrombus. MRV test results with the cause of hypoplasia have 100% sensitivity, 67.4% specificity, and 70% accuracy. Meanwhile, we found that 41% sensitivity, 82% specificity, and 50% accuracy because of thrombus.

Conclusion: There was significant compliance between 3D-TOF MRV cerebral findings along with cerebral angiography, especially at thrombus.

Keywords: Magnetic Resonance Venography, Cerebral Angiography, Thrombus, Nonvisualized

Introduction

The use of Magnetic Resonance Venography (MRV) cerebral as a non-invasive method in the diagnosis of intracranial venous system abnormalities start to increase. MRI combined with MR Venography (MRV) has largely replaced the conventional invasive modalities such as angiography and CT. MRV could be done without the use of contrast agents but by using of Time-of-Flight (TOF) technique. However, based on the previous studies, flow and artifacts could interfere in the evaluation of venous structures.

MRV has been widely used because not only it is non-invasive but also it does not give radiation exposure like cerebral angiography and CT Venography procedure. Therefore, the non-visualized (vein image not visualized) findings on MRV often led to diverse interpretations. Some previous studies also found many images that show MRV pitfall or inconsistency in findings compared with cerebral angiography as the
gold standard: R.H. Ayanzen et al. (31%), Sharma UK (47%), E. Widjaja (51%) 4.

There has been few research findings comparing MRV with cerebral angiography so far. The aim of this study is to evaluate the findings of non-visualized on MRV compared to cerebral angiography as the gold standard to help the interpretation of the examinations to avoid misdiagnosis.

Method

Observational retrospective analytic study was used in this study that conducted at Husada Utama Surabaya Hospital from October 2013 to February 2014. The data obtained from observing the medical records of patients that examined with MRV cerebral and angiography cerebral 5. There were 49 patients that examined with MRV cerebral and angiography cerebral. However, we only used 39 samples which their medical records were completed and accessible. Interpretation of the results of MRV and cerebral angiography performed by two specialists in radiology meanwhile, the data were analyzed using SPSS 6.

Results

Most of the research samples were chronic CVA patients (35.89%) while, patients with a ruptured aneurysm (2.56%) were the fewest sample. The data distribution of patients based on clinical diagnosis could be seen in Table 1. Meanwhile, table 2 shows the location of abnormalities with the highest percentage was in the transverse sinus (35.45%), and the lowest percentage was in the superior sagittal sinus (1.27%).

The examination of 39 samples using MRV found 50 findings. Detailed MRV examination results could be seen in Table 3. MRV examination results show the highest readings was hypoplasia in 19 people (38%), while the thrombus and non-visualized respectively were 18 people (36%) and 13 patients (26%).

We found 50 findings in 39 patients using angiography. The results of angiography could be seen in Table 4 that was showed the highest result in the examination is thrombus in 39 samples (78%). Table 5 shows the results of cross tabulation between MRV examination results with the results of angiography examination. 20 samples (40%) from the results of MRV examination are found match with the results of angiography. MRV examination results with hypoplasia were 19 samples, while the results of angiography were only 4 samples (21%). The remaining 11 samples were thrombus and four other samples were normal. MRV examination results showed 18 samples of thrombus while, 16 samples (88.9%) of thrombus were found in the results of angiography. Then, the remaining two samples were normal. MRV examination results showed 13 samples of non-visualized which examined with angiography showed 12 samples (92.3%) of thrombus and the other one sample was normal.

Table 5 shows the results of MRV sensitivity of hypoplasia were 100% with a specificity of 67.4% and accuracy of 70%, whereas the results of MRV sensitivity of the thrombus were 41% with a specificity of 82% and accuracy of 50%. Both MRV and cerebral angiography readings in this study were observed by the two senior radiology specialists (dr. ADS experts of neuroradiology and dr. LM who works in interventional radiology section). If there is a difference in the interpretation of MRV and cerebral angiography, then decisions are taken by consensus (agreement). However, before consensus, each observer of MRV and cerebral angiography were tested their interpretation reliability by calculating the interobserver variation for each parameter characteristic image of MRV and cerebral angiography that were observed. The interobserver reliability showed good results, with a kappa coefficient (k) above 0.9 with p <0.05 for all parameters examined in the interpretation on the readings of MRV and cerebral angiography. It could be said that the examiners have good reliability interobserver.
Table 1. Data Distribution Based on Clinical Diagnosis

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vertigo</td>
<td>7</td>
<td>17.95</td>
</tr>
<tr>
<td>Cephalgia</td>
<td>2</td>
<td>5.13</td>
</tr>
<tr>
<td>Chronic CVA</td>
<td>14</td>
<td>35.89</td>
</tr>
<tr>
<td>Acute CVA</td>
<td>10</td>
<td>25.64</td>
</tr>
<tr>
<td>Sub-acute CVA</td>
<td>3</td>
<td>7.69</td>
</tr>
<tr>
<td>TIA</td>
<td>2</td>
<td>5.13</td>
</tr>
<tr>
<td>Ruptured aneurysm</td>
<td>1</td>
<td>2.56</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 2. Distribution of Data Based on Location Abnormalities

<table>
<thead>
<tr>
<th>Abnormalities location</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superior sagittal sinus</td>
<td>1</td>
<td>1.27</td>
</tr>
<tr>
<td>Straight sinus</td>
<td>2</td>
<td>2.53</td>
</tr>
<tr>
<td>Sigmoid sinus</td>
<td>26</td>
<td>32.91</td>
</tr>
<tr>
<td>Transversus sinus</td>
<td>28</td>
<td>35.45</td>
</tr>
<tr>
<td>Jugular vein</td>
<td>22</td>
<td>27.84</td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 3. Data Distribution Based on Inspection Results MRV

<table>
<thead>
<tr>
<th>Result of MRV examination</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hipoplasia</td>
<td>19</td>
<td>38</td>
</tr>
<tr>
<td>Trombus</td>
<td>18</td>
<td>36</td>
</tr>
<tr>
<td>Non visualized</td>
<td>13</td>
<td>26</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 4. Data Distribution Based on Inspection Results Angiography

<table>
<thead>
<tr>
<th>Result of Angiography</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hipoplasia</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Trombus</td>
<td>39</td>
<td>78</td>
</tr>
<tr>
<td>Normal</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 5. Cross-Tabulation Results of MRV with Angiography

<table>
<thead>
<tr>
<th>Hipoplasia</th>
<th>Angiography</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trombus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-visualized</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Based on the data, the distribution pattern of sample by the highest amount of clinical diagnosis are chronic CVA 14 people (35.89%), vertigo 7 people (17.95%), cephalgia 2 people (5.13%), acute CVA 10 people (25.64%), sub-acute CVA 3 people (7.69%), TIA 2 (5.13%). The findings are consistent with the previous studies that have reported CVT could be found in about 10-20% of stroke patients. The distribution based on the
highest percentage abnormality located on the first place were; transverse sinus abnormalities 35.45%, sigmoid sinus 32.91%, jugular venous 27.84%, straight sinus 2.53%, and superior sagittal sinus 1.27%.

Location prediction is matching with previous research that showed 31% of abnormalities found in sinus transversus and 90% of thrombus have been reported in transverse sinus and sigmoid sinus, while 15% to 30% of non-visualized patients have partial or complete absence on one of the transverse sinus. Images related the loss signal of blood vessels mostly appeared at the use of 3D-TOF MRV that occurs in sinus transverse non-dominant. If it appeared on the sinus transverse dominant, it could be estimated that there are venous obstruction which resulting in the appearance of venous hypertension intracranial. Variant of normal venous anatomy may resemble sinus thrombosis 7. MRV artifacts could resemble cerebral venous thrombosis in the absence of normal signal flow in venous or sinus 8.

Test results of MRV with hypoplasia were 19 samples, but the results of angiography with hypoplastic were only 4 samples (21%), while the remaining 11 samples thrombus and four other samples were normal. That happened may be due to changes in the dynamics of blood flow in hypoplasia or aplasia venous sinus resulting in loss of flow marks on 3D-TOF MR Venography which able to resemble thrombosis. MRV pitfall in the diagnosis is related to flow and refocusing of the slow flow 9, that could resemble intraluminal thrombus or blood products paramagnetic (intracellular deoxyhemoglobin or methemoglobin) as a normal signal 10. Low T2 signal intensity in the early stages could be interpreted as a flow-void 10. This variant could be further divided into veins anatomical variants that resemble occlusion (sinus atresia or hypoplasia), asymmetric or sinus drainage variant (occipital sinus, sinus duplication), and normal sinus filling defects (arachnoid granulation, intrasinus septa). Usually, when there is a low signal into a vein, it is associated with flow void and signs of venous patency 11. However, there are intracellular deoxyhemoglobin, which is dark on T2 and resembles a flow void at some stages of thrombus. Hypoplasia and aplasia transverse sinus on the right or left side is a common finding that could be considered as sinus thrombosis because on the MRA showed one of the transverse sinuses was not visible. If hypoplasia still suspected on the transverse sinus, the size of the jugular foramen must be observed 12.

MRV test results showed 18 samples of thrombus, from 18 samples to 16 samples (88.9%) of thrombus and the other two samples were normal on cerebral angiography examination. Results of previous studies showed that MRV has sensitivity of 100% and a specificity of 78.6% for detecting the presence of thrombus. High sensitivity of MRV to see a thrombus in the thrombus images is due to dural sinus or vein is pathognomonic. Signals of thrombus within the dural sinus or cortical veins vary. However, most patient’s thrombus image showing of hyper-intense signal in both the T1 and T2 images. Abnormal T2 signal replaces the normal signal of sinus thrombosis as a definite sign of the existence of a very slow blood flow that could sometimes create a high signal on spin echo T1WI. Findings on the examination of two samples using MRV were read as thrombus but in cerebral angiography read as normal, this may occurs because MRV artifacts may resemble cerebral venous thrombosis in the absence of signals in normal flow or venous sinuses. Most of the loss of artifactual on vascular signal could be seen with the use of 3D-TOF MRV 13.

MRV test results showed 13 samples of non-visualized, from those 13 samples the angiography obtained 12 samples (92.3%) of thrombus, while the remaining sample was normal. Findings nonvisualized on MRV showed 92.3% of thrombus on its angiography cerebral could be due to the 3D-TOF flow gap occurs as a result of intravascular slow blood flow as blood flow patterns are complex and varied features 14. This diversity illustrates the pathophysiological changes seen after venous occlusion. There are two main processes; the first process is the presence of local edema and venous infarction that usually seen after a cortical vein occlusion, while the second process is the presence of intracranial hypertension, both processes often occur together 15. These results indicate that the sensitivity of hypoplasia using MRV of 100% with a specificity of 67.4% and accuracy of 70%, whereas the sensitivity of the thrombus using MRV is 41% with a specificity of 82% and accuracy of 50%.

Conclusions

MRV test’s results have compatibility with cerebral angiography examination’s results by 40%. The examination results MRV suitability with the results of angiography showed 21% hypoplasia, 88.9% thrombus. Meanwhile, MRV test results that showed nonvisualized obtained 92.3% of thrombus while the remaining 7.7%
were normal in the results of angiography.

MRV test results with the cause of hypoplasia have a sensitivity of 100% and a specificity of 67.4% and an accuracy of 70%. The examination results of MRV with the cause of thrombus have a sensitivity of 41% with a specificity of 82% and an accuracy of 50%. MRV examination without contrast has a diagnostic value that could be considered as non-invasive imaging methods that are useful in cerebral venous disorders.

**Ethical Clearance:** This research involves participants in the survey using sampling method that was accordant with the ethical research principle based on the regulation of research ethic committee. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, nonmaleficence, and justice.

**Conflict of Interest:** The authors report that there is no conflict of interest related with this paper.

**Source of Funding:** This study is done with authors’ funding only.

**Authors’ Contribution:** Arif Shidiq do the laboratory research while Anggraini Dwi Sensusiti writes the laboratory result into this paper.

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Investigating the Effect of Short-Term Educational Program on Readiness of Patient’s Candidate for Endoscopy

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Abstract

Background: Gastrointestinal endoscopy is one of the main diagnostic tools for gastrointestinal examination. In this study, we measured the effect pre-procedure education on the readiness of patients for the endoscopy.

Method: This quasi-experimental study was performed on 70 patients aged 35-60 years who were candidates for endoscopy from SEP 2018 to MAY 2019. Data were collected by a valid and reliable, 22 items’ questionnaire that developed by the researcher. Data were analyzed by SPSS, version 16.

Results: mean score of readiness for endoscopy in the intervention group 105.8 (5.3) was significantly higher than control group 62.6 (7.8), (p<0.001). Also, the level of readiness of the intervention group was 11.4% good and 88.6% very good. The level of readiness of the control group was 91.4% moderate and 8.6% good.

Conclusions: Considering the effect of pre-procedure education on the readiness of patients for the endoscopy, it can be inferred that providing educational programs before endoscopy can be helpful.

Keywords: readiness, endoscopy, anxiety

Introduction

Gastrointestinal endoscopy is one of the main diagnostic tools for gastrointestinal examination. This diagnostic method, which is frequently used in clinical settings, has obvious diagnostic benefits and therapeutic applications(1). Patients who are candidates for endoscopy should receive the necessary information about readiness for endoscopy accurately and simply because of the need for complete gastrointestinal clearance for proper physician visibility and non-repetition of endoscopy. On the other hand, the findings of a study examining pre-procedure anxiety showed that the level of anxiety was severe in 14% of patients and moderate in 74% (2).

Asymmetry reduces patients’ health(3). Anxiety is especially important in gastrointestinal patients(4). Therefore, the role of patients’ physical and mental readiness is crucial to achieve the expected outcome of endoscopy in terms of correct and timely diagnosis and treatment, with minimal damage to and side effects on the patient (5, 6). Readiness is the quality or status of preparation for the procedure (7).

Readiness for endoscopy increases the likelihood of successful endoscopy and prevention of repeating endoscopy as well as early diagnosis of the disease (8). Research on medical complaints has shown that the most common form of neglect has been reported due to the patient’s lack of readiness for interventions (9). It can be expected that if patients candidate for endoscopy receive empathetic information, they will cooperate better, accept the process consciously, and endure the pain and possible side effects more easily (6, 10, 11). In this study, we measured the effect pre-procedure education on the...
Material and Method

The sample was randomly selected from the population of endoscopy candidates who admitted in Sheikh Mostafa hospital, and assigned to control and intervention group. Each group consist 35 volunteer patients. Inclusion criteria were age between 35-60 years, full consent to participate in research, having acceptable literacy to write and read, no previous history of endoscopy, no known mental illness and anxiety disorders, no emergency conditions for endoscopy, no history of drug and alcohol use, lack of effective visual and hearing impairment, and exclusion criteria were emergency conditions such as hemorrhage and rupture during endoscopy, as well as patients’ unwillingness to participate in the study.

The instrument used in this study was the endoscopy readiness questionnaire. The first part of the questionnaire included demographic information including age, gender, job, education, income, marital status, and the second part was the endoscopy readiness questionnaire was developed that assesses patients’ readiness in the areas of information provision, mastery of endoscopy team, anxiolysis (de-stressing), up close experience, and training during endoscopy. To assess content validity of instrument using the Waltz & Bausell method (12), the primary 27 items questionnaire were evaluated by 10 experts including internist and experienced nurses in terms of relevance, clarity, and simplicity. According to experts’ comments, the three lowest-scored items were deleted, and several of them were combined and modified and finally a 22-items questionnaire was prepared (table 1). Last item of the questionnaire did the open-ended question that asked the patient viewpoint for likelihood of re-select the center for doing later endoscopies. Based on a Likert scale, each item scored in a range from 1 (Strongly disagree) to 5 (Strongly agree) total score ranged from 21 as lowest to 105 as highest readiness.

To determine the reliability of the questionnaire, a test- retest method was used. The questionnaire was administered to 10 candidate patients for endoscopy who were not included in the intervention or control group, and two weeks later the same subjects completed the questionnaire again. The test-retest correlation coefficient ($r = 0.97, 0.001$) indicated the reliability of the instrument. Indeed, a value of 0.949 was obtained for Cronbach’s alpha which was an acceptable score to confirm the internal consistency of the questionnaire.

Data were analyzed using SPSS, version 16. Mean and standard deviation were used for descriptive statistics, and chi-square and t-test were used for inferential statistics.

Findings

Kolmogorov-Smirnov test and showed that the two groups were homogeneous in terms of demographic characteristics and there were not significant differences. The frequency of respondents to each item of the questionnaire is listed in Table 2. the mean scores of endoscopy readiness after for intervention group (90.8±5.3) was more than control group (50.6±7.8) significantly ($p <0.001$) (Table 3). In terms of comparing the levels of readiness, there was a statistically significant difference between the two groups (Table 3). Regression analysis showed that in the intervention group, 11.4% of participants had high level of readiness and 88.6% had very high level of readiness. In the control group, 91.4% of participants had moderate level of readiness and 8.6% had high level of readiness. Also, in response to the open-ended question, 81% of the patients in the
intervention group stated that they would choose the same center for future referral, compared to 38% in the control group.

**Table 1. Distribution of demographic characteristics by study groups**

<table>
<thead>
<tr>
<th>variable</th>
<th>Intervention group</th>
<th>Control group</th>
<th>Z score</th>
<th>p- value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No (%)</td>
<td>No (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Man</td>
<td>22(62.9)</td>
<td>19(54.3)</td>
<td>0.384</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Woman</td>
<td>13(37.1)</td>
<td>16(45.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Job</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed (employee, self-employment)</td>
<td>24(68.6)</td>
<td>22(62.9)</td>
<td>0.420</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Unemployed (unemployed, disabled, retired)</td>
<td>11(31.4)</td>
<td>13(37.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Degrees under diploma and diploma</td>
<td>6(17.1)</td>
<td>14(40)</td>
<td>0.387</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Associate degree and above</td>
<td>29(82.9)</td>
<td>21(60)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Income</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Up to one 200$ monthly</td>
<td>29(82.9)</td>
<td>14(40)</td>
<td>0.449</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Over 200$ monthly</td>
<td>6(17.2)</td>
<td>21(60)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>30(85.7)</td>
<td>28(80)</td>
<td>0.503</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Single and dead spouse</td>
<td>5(14.3)</td>
<td>7(20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean and standard deviation of age (years)</td>
<td>50/49(±7/17)</td>
<td></td>
<td>0.121</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

**Table 2: Mean endoscopy readiness score in two groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Mean (SD)</th>
<th>Mean difference</th>
<th>95% confidence interval for mean difference</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intervention</td>
<td>35</td>
<td>90.8(5.3)</td>
<td>42.2</td>
<td>46.3-39.9</td>
<td>26.95</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Control</td>
<td>35</td>
<td>48.6(7.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 3: Readiness levels of intervention and control groups**

<table>
<thead>
<tr>
<th>Variable Number</th>
<th>Intervention</th>
<th>Control</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Readiness for endoscopy</td>
<td>Percentage</td>
<td>Number</td>
<td>Percentage</td>
</tr>
<tr>
<td>Moderate</td>
<td>0</td>
<td>0</td>
<td>32</td>
</tr>
<tr>
<td>High</td>
<td>4</td>
<td>11.4</td>
<td>3</td>
</tr>
<tr>
<td>Very high</td>
<td>31</td>
<td>88.6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Discussion

According to the results of the study, the post intervention mean score of readiness of the patients in the intervention group was significantly higher than the control group, which indicates the effectiveness of education. Findings of Beitzer study (10), which aimed to investigate the effect of film education on the level of satisfaction and anxiety of patients undergoing colonoscopy, showed that education leads to more tolerance and patient satisfaction, which is in line with the results of the current study. Patients who are candidates for endoscopy should receive the necessary information about readiness for endoscopy accurately and simply because of the need for complete gastrointestinal clearance for proper physician visibility and non-repetition of endoscopy. Failure to receive this information increases patients’ anxiety (13). In a study by Onal et al. in Turkey, it was confirmed that a lack of awareness of the treatment process was a source of increased anxiety among patients and subsequently an increase in the need for sedative medications (14). In this vein, the results of a qualitative study conducted by Azami et al. (2016) to examine the expectations and experiences of patients with angioplasty suggest that lack of knowledge about the procedure and its side effects has been one of the main themes, and this defect in patient education was caused mainly by the care team (13). Pehlivian et al. (6), reported that the benefits of providing information to candidate for anxious therapies such as endoscopy are increasing patients understanding and facilitating patients’ coping with endoscopy which have been shown to achieve more successful outcomes.

Moyo et al. stated that the main causes of dissatisfaction and failure to reach therapeutic goals are lack of information about the disease process and interventions performed by the care team, lack of attention to the patient’s and family’s ambiguities, and failure to provide meaningful explanations to the patient (15). Pre-endoscopic education can cause success in therapeutic interventions, increase satisfaction, reduce nurses’ workload as well as reduce the cost for patients because of the prevention of endoscopy repetition due to the failure of the physician to obtain proper vision, and accelerate patients’ recovery. The results of some studies confirming this finding indicated the effects of different methods such as patient preparation (11), providing clear information and social support (16), providing cognitive and behavioral information (17), nursing counseling (18), using Quran phonics (19) and informing patients before endoscopy (9) on the success of therapeutic procedures such as endoscopy.

Conflict of Interest: There is no conflict of interest between authors.

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Ethical Clearance: Ethics Code IR.MEDILAM.REC.1397.156

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In silico Analysis of “Interferon Beta 1” In some Selected Animal Species

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Abstract

Type I interferons (IFNs), are considered as a main host immune system cytokines effector against infectious virus. In addition to innate immune receptor bacterial ligands and/or bacterial infections. Furthermore, the development of kinds IIIFNs are also triggered, suggesting a wider physiological function for those cytokines in homeostasis and host protection than initially expected. Results: To recognize both structural divergence and sequence various bioinformatics methods were used. Initial sequence analysis of IFNß1 showed that they shared with human over 70% similarity and some identity like Rhesus monkey, pig, domestic cat, for dog. With few exceptions, sequences showed a high degree of sequence preservation. The physico-chemical analysis indicated a large developmental difference between humans and others and an estimate of 39% to 42% of hydrophobic residues. Four human mammals house mouse pig Norway rat have various Pfam types. The secondary structure of IFNß1 composed of random coil, extended strand and alpha helix. Conclusions: Depending on the results collected, which maybe assumed that in these mammalian species IFNß1 has the same counterpart, highly retained and functional similarities.

Keywords: Interferons, host protection, cytokines, divergence, counterpart.

Introduction

Type I interferons (IFN) play an essential role in the defense of antivirals and are formed in a variety of cells following viral infection. It is understood that IFN-a/b therapy of immature dendritic cells (DC) causes their phenotypic and functional maturation. Interferons (IFNs) are a group of cell-produced pleiotropic cytokines in reaction to viral infections [1]. Such cytokines have antiviral, immunomodulatory, and antitumor properties by regulating the expression of hundreds of genes involved in critical biological processes such as progression of the cell cycle, cell proliferation, and apoptosis [2]. Some cells, such as endothelial cells, osteoblasts, action, lymphocytes, fibroblasts and macrophages (NK cells, T-cells and B-cells) topathogens, are secreting IFNßs. Through expression of several genes, they enable NK cells and MFs to elicit immunomodulatory, antiviral, antitumor, and anti-inflammatory responses [3] including response to therapy. A number of disease modifying drugs, including traditional first line agents such as, interferon-beta (IFN-ß).
Materials and Method

2.1 Recovery or analysis of sequences

Nucleotides and amino acid sequences of Mus musculus (house mouse), Homo sapiens (human), Rattus norvegicus (Norway rat), Sus scrofa (pig), Macaca mulatta (Rhesus monkey), Felis catus (home cat) and Canis lupus familiaris (dog) have been extracted from NCBI. For obtain related sequences in other species, the Basic Local Alignment Selection Tool (BLAST) was used. Seven IFNB1 protein mammalian species were considered for study. NCBI (nlm.nih.gov/bank) obtained three classifications in fastfor mal.

2.2 Determination of personality and similarity percentage

The percentage of identity and similarity in domestic cat, human, dog, house mouse, Norway rat, pig and Rhesus monkey among the amino acid series of the IFNB1 genes were identified by showing a pair sequence comparison using BLAST.

2.3 IFNB1 Protein Physicochemical Properties Determination

“The proteomic database of the (SII/B) (wlb. exlpasy. orl), to use the (ExPASy), physicochemical properties of the seven mammalian species IFNB1 protein was defined. Protein analysis was performed on the web server PepTide 21.10 (http://peptide2.com/lnp eptidehydrophobicityhydrophilicity.phlp), whereas humans were usledals references points.”

Results

IFNB1 gene sequences of nucleotides and amino acids recovered:

Variations were shown in the distances of the amino acid sequences and retrieved nucleotide. The length of the IFNB1 gene nucleotide sequences ranged from 555-839 bps while the amino acid sequence length differed from residues of 182-187 amino acids. Among the seven selected mammals, thllegenlhovfthle human IFNB1 nucleotide sequences were the longest (839bps), followed by the house mouse (750bps) and the shortest (555bps) of the Norway rat. The residues of amino acids were the same for Rhesus monkey and human (187). 186amino acid residues respectively, in pig, dog and domestic cat. Residue of shorter amino acids for house mouse (Table 1).

Table 1. The distances of the amino acid sequences and retrieved nucleotide.

<table>
<thead>
<tr>
<th>Species</th>
<th>Gene name</th>
<th>Gen accession</th>
<th>base pair (bp)</th>
<th>Amino acid length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homo sapiens human</td>
<td>interferon beta 1 IFNB1</td>
<td>NP_002167</td>
<td>839</td>
<td>187</td>
</tr>
<tr>
<td>Mus musculus house mouse</td>
<td>interferon beta 1 IFNB1</td>
<td>NP_034640</td>
<td>750</td>
<td>182</td>
</tr>
<tr>
<td>Sus scrofa pig</td>
<td>interferon beta 1 IFNB1</td>
<td>NP_001003923</td>
<td>561</td>
<td>186</td>
</tr>
<tr>
<td>Rattus norvegicus Norway rat</td>
<td>interferon beta 1 IFNB1</td>
<td>NP_062000</td>
<td>555</td>
<td>184</td>
</tr>
<tr>
<td>Macaca mulatta Rhesus monkey</td>
<td>interferon beta 1 IFNB1</td>
<td>NP_001129267</td>
<td>564</td>
<td>187</td>
</tr>
<tr>
<td>Felis catus domestic cat</td>
<td>interferon beta 1 IFNB1</td>
<td>NP_001009297</td>
<td>561</td>
<td>186</td>
</tr>
<tr>
<td>Canis lupus familiaris dog</td>
<td>interferon beta 1 IFNB1</td>
<td>NP_001129259</td>
<td>561</td>
<td>186</td>
</tr>
</tbody>
</table>
Physicochemical properties of IFNB1 protein:

The IFNB1 protein analysis of selected mammals showed, when compared, changing physicochemical things. The result exposed that pig IFNB1 protein (4.93) was the least theoretical pl, while house mouse IFNB1 protein (9.69) was the highest. The molecular weight of IFNB1 proteins from the seven mammalian species showed that the weight of human IFNB1 protein was 22293.88 kDa, while the weight of dog IFNB1 protein was 22387.92 kDa. The weight of the pig protein was 21950.46 kDa, the least. The percentages hydrophobic residues in IFNB1 protein of the species showed the following: human (39.57%), house mouse (42.31%), domestic cat (40.32%). The Norway rat (40.76%), Rhesus monkey (40.64%) and pig (42.47%) higher percentage (Table 2).

Table 2. IFNB1 protein analysis selected mammals showed, compared, changing physicochemical things.

<table>
<thead>
<tr>
<th></th>
<th>human</th>
<th>house mouse</th>
<th>pig</th>
<th>Norway rat</th>
<th>Rhesus monkey</th>
<th>domestic cat</th>
<th>dog</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of amino acids</td>
<td>187</td>
<td>182</td>
<td>186</td>
<td>184</td>
<td>187</td>
<td>186</td>
<td>186</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>22293.88</td>
<td>22126.77</td>
<td>21950.46</td>
<td>22072.56</td>
<td>22315.93</td>
<td>22187.64</td>
<td>22387.92</td>
</tr>
<tr>
<td>Theoretical pl</td>
<td>8.93</td>
<td>9.69</td>
<td>4.93</td>
<td>9.73</td>
<td>8.76</td>
<td>6.44</td>
<td>5.86</td>
</tr>
<tr>
<td>Instability index</td>
<td>47.91</td>
<td>37.93</td>
<td>62.91</td>
<td>37.43</td>
<td>47.75</td>
<td>42.90</td>
<td>51.85</td>
</tr>
<tr>
<td>Aliphatic index</td>
<td>99.63</td>
<td>92.14</td>
<td>99.57</td>
<td>91.63</td>
<td>95.99</td>
<td>96.94</td>
<td>104.84</td>
</tr>
<tr>
<td>Hydrophobic residues</td>
<td>39.57%</td>
<td>42.31%</td>
<td>42.47%</td>
<td>40.76%</td>
<td>40.64%</td>
<td>40.32%</td>
<td>39.78%</td>
</tr>
<tr>
<td>Acidic residues</td>
<td>9.63%</td>
<td>8.79%</td>
<td>13.44%</td>
<td>8.15%</td>
<td>10.16%</td>
<td>12.37%</td>
<td>13.44%</td>
</tr>
<tr>
<td>Basic residues</td>
<td>14.97%</td>
<td>15.38%</td>
<td>10.75%</td>
<td>14.67%</td>
<td>14.97%</td>
<td>13.98%</td>
<td>13.98%</td>
</tr>
<tr>
<td>Neutral residues</td>
<td>35.83%</td>
<td>33.52%</td>
<td>33.33%</td>
<td>36.41%</td>
<td>34.22%</td>
<td>33.33%</td>
<td>32.8%</td>
</tr>
</tbody>
</table>

IFNB1 gene secondary protein structures:

For the seven chosen mammalian species, GORIV software was used to predict the secondary structures of IFNB1 protein. Their IFNB1 protein is shown to contain mainly random coil, extended strand and alpha helix. Though, the human IFNB1 protein alpha helix was 43.85 percent, while mammals were 69.78 percent higher for house mouse. The least was in the pig with 41.94% of the design in alpha helix. In addition, the IFNB1 protein expanded strand for humans was the longest (23.53%) with the dog being the shortest (10.22%). Variations have been found on the unexpected coils of the seven species additional structures of IFNB1 protein with the random coil of pig IFNB1 protein occupying 41.94% of the structure relative to other mammals (Table 3).
Discussion

Type I interferons (IFN) are a group of cytokines expressed under physiological conditions at low levels or induced by stimuli such as viral infection to high levels[7]. Form I IFN includes a number of evolutionary proteins produced by closely related and connected genes, the major species being several IFN-a subtypes and a single IFN-b [8]. Here we note that sequence lengths of nucleotide amino acids varying from the human, house mouse, pig Norway rat, Rhesus monkey, domestic cat, and dog gene IFNB1. It is also claimed that variations in the sequence length are induced by mutations in the indels, which may have collected through evolution.

As a results, the percentage of human, house mouse, pig, Norway rat, Rhesus monkey, domestic cat and dog identity ranged from 48.7-95.2 percent compared to that of human IFNB1 amino acids, viewing that they might have related evolutionary strategies. This is also the situation with percentage similarity (62-97.9 %). Importantly, sequences of approximately 70 percent percent similarity indicate the IFNB1 gene has the same homology, similarities of function And the preservation is very high. In addition, Xu and Joshi [9] reported that if two samples have a sequencing similarity higher than 70%, it is proposed that they have been inclined to share approximately 90% or more of the same functions and biological processes. Proteins in the samlegroluphalve a sequencessimilarity of at lastmolorethahn 30 % of alminoacilds. “In clinicians with relapsing multiple sclerosis[10][11] and in patients with chronic hepatitis C infection virus [12][13]. IFNβ treatment significantly improved the rate and suppressive activity of TReg cells, and also decreased expression of Foxp3 mRNA in PBMCs in patients with recurrence-remitting multiple sclerosis[14][15].

“NLRP1 and NLRP3 inflamasomes are directly inhibited by IFNβ signaling) in an STAT1-dependent manner. Second, IFNs of type I induced IL-10 production which infrared effect actuated the STAT3 transcription factors in n an autocrinemanner[16]. Type I IFNs was tested as IBD therapy. Although some medical therapy studies initially showed promising results in patients with ulcerative colitise[17][18].”

It may depend on the conduct’ motives. Thus stressing the adequacy of the phylogenetic tree, the mammalian species separation period represents Stone et al previous reports. 28.

“GRAVY value greater than zero, according to Kyte and Doolittle[19] indicated a enzyme very hydrophobic. Though, for seven mammals IFNB1 proteinThe values of GRAVY gained in the present study are less than zero, implying that they are in nature rather hydrophilic.”

In all the seven mammalian species mentioned in this study, the fact that IFNB1 protein has lower damagingly exciting remains than positive exciting remains renders IFNB1 proteins intracellular in these animals[20]
According to Guruprasad et al, the coefficient of validity, that is the measurement of a protein stability in vitro when it reaches NMT sequences and 3D structures has revealed motif properties in addition to the known PROSITE motif that are utilized in a new predictor described here. The composite prediction function (with separate ad hoc parameterization) indicates that the protein may be safe. This is attributed, as stated by Devi et al., to the surplus tyrosine that results in the structure of the disulfide bonds relationship in the protein molecule. Regrettably, we show a measurement of validity greater than 50, suggesting that this protein is likely to be in vitro defective. Our finding revealed that secondary elements on the mammalian species subordinate structures of the seven IFNB1 protein were alpha-helix, expanded strand and the spontaneous coils. These are the three mechanisms were involved in the protein folding stability and function.

Conclusion

Structural analysis and sequence of seven mammals indicated regions correlated with evolution and structure. In addition to the variation of retained amino acid sequences from the physico-chemical properties in the active site, preservation may lead to the functional heterogeneity in a few amino acid substitutions at the respective site. Because IFNB1 has a role in immunomodulatory, antiviral, anti-tumor effects are very significant inflammatory protein conditions, the studies on it will lead to further research into proteomics.

Conflict of Interest: There is no conflict of interest among the authors.

Funding: Self

Ethical Clearance: This study is ethically approved by the Institutional ethical Committee.

References


Rapid Qualitative Test for Drunkenness Detection and the Presence of Alcohol in Beverages

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Abstract

In many situations like car accidents, troubles and crime scenes we need to know if someone is drunk. This research includes rapid test to detect the alcohol drinking for those that not exhibit any signs of drunk like ataxia and vomiting, and to detect the beverage containing alcohols. The study included 30 volunteers of alcoholics and five types of beverages (Whisky, vodka, beer, wine and barbican). The sampling includes random quantity of sputum and 2 ml of blood from the volunteers. The sputum and beverages are examined by qualitative method depending on the change of dichromate solution color which is saturated and dried up on a filter paper piece in polyethylene container ready for use. If the color changes from yellow to blue-green this means that the sample contains alcohol and the result is positive, and if the color of dichromate dose not changed the result is negative and the sample dose not contains alcohol. The blood samples were examined by the measuring of GGT enzyme to confirm alcohol drinking. All of the sputum samples showed positive results by changing the color of dichromate. All of the beverages except barbican showed positive results this means that these beverages contain alcohol while barbican not. The values of GGT for volunteers were higher than the normal values which confirm the test of sputum.

Key words: Alcoholics, beverages, sputum and potassium dichromate.

Introduction

Alcohols are organic compounds which contain hydroxyl functional group (OH) connected to carbon atom.¹ The term alcohol belongs to primary alcohol ethanol which is the predominant type of alcohol in beverages produced by fermentation of sugars via yeasts². Alcohols are neurotoxic and psychoactive drugs.³ Alcohols can cause alcohol intoxication when consumed in large amounts. Ethanol is colorless, flammable, liquid, light chemical odor with chemical formula CH₃CH₂OH which is abbreviated to C₂H₅OH⁴. Ethanol has many uses like antiseptic⁵, antitussive⁶, antidotes⁷, chemical solvent⁸ and recreational⁹. Although alcohol has many benefits, its drinking causes adverse effects like ataxia (10), gastrointestinal diseases (11), cancer¹² and dependence¹³. In human body ethanol is metabolized to acetaldehyde by alcohol dehydrogenase enzyme in liver and then oxidized by acetaldehyde dehydrogenase enzyme to acetyl Co-A which is the final product of fat and carbohydrate metabolism and can be used to produce energy. Ethanol is a macronutrient energetic molecule yields about 7 kcal.per gram¹⁴. The first product of ethanol metabolism acetaldehyde is more toxic than ethanol¹⁵. Acetaldehyde showed to increase the risk of developing cirrhosis and multiple forms of cancers¹⁶. When ethanol is metabolized by dehydrogenase liver enzymes NAD will be converted to reduced NAD, normally, NAD participates in the fat metabolism in the liver; therefore, alcohol competes with fat for NAD. Long term alcohol drinking leads to fat accumulation in the liver causing fatty liver. Alcoholism leads to cell death in the hepatocytes because the stored fat reduces the function of the cell. These cells are then converted to scar tissue leading to cirrhosis, Sherwood et al¹⁷. Liver can metabolize certain amounts of alcohol, when the consumed alcohol exceeds the ability of liver to metabolize, alcohol reaches the heart and reduces the force of heart contraction. Consequently, the heart will pump less blood, lowering the total body blood pressure. When alcohol reaches the heart it also reaches the lungs, in this stage the person can breathe out trace of alcohol, this is the major principle of alcohol breath testing to determine if a driver has been drinking and drive¹⁸.
Materials and Methods

Principle

Alcohol reacts with yellow potassium dichromate (K₂Cr₂O₇) to produce blue-green Chromium (III) sulfate. Therefore the appearance of blue-green color means the presence of alcohol when a solution is added to potassium dichromate as shown below:\(^1\)

\[
2\text{K}_2\text{Cr}_2\text{O}_7 + 8\text{H}_2\text{SO}_4 + 3\text{CH}_3\text{CH}_2\text{OH} \rightarrow 2\text{Cr}_2(\text{SO}_4)_3 + 2\text{K}_2\text{SO}_4 + 3\text{CH}_3\text{COOH} + 11 \text{H}_2\text{O}
\]

Orange-yellow alcohol blue-green

Preparation of test solution

In a clean dry 250 ml beaker add 5.0 ml of 0.25 M potassium dichromate (K₂Cr₂O₇) solution, add 1 drop of 0.1 M silver nitrate (AgNO₃) and swirl the beaker immediately after addition, add 5.0mL of 6 M sulfuric acid (H₂SO₄) and swirl the beaker immediately after addition. The color of this test solution is orange – yellow which is converted to blue – green after the addition to alcohol or the addition of any solution containing alcohol.

Technique:

Saturate a filter paper with test solution prepared above for about 3 hours then dry this filter paper by using drying oven on 30°C until it be dry. The filter paper now is dry orange color containing the test solution; the filter paper is only holder for test solution. Cut the filter paper in a circle shape in a diameter of 3 cm and put it in plastic translucent cylindrical container with a volume of 10 ml and cap the container. The plastic container now is ready for use.

Sampling:

The samples that are examined are sputum, blood, and beverages. The sputum and blood were collected from 30 volunteers, while the beverages (Whisky, vodka, beer, wine and barbican) were purchased from markets. The sputum samples and beverages have been examined by potassium dichromate test, while the blood samples are used to determine the concentration of GGT enzyme as confirmatory test of alcohol drinking. Sputum of non alcoholics and water were used as a control to be compared with alcoholics and beverages respectively.

Test procedure

1. Open the cap of the plastic container that contains the test solution filter paper.
2. Add the sample under test in a quantity of 1 – 3 ml.
3. Recap the plastic container and shake it gently for 5 minutes.
4. Notice the color change and record the result.
5. You can add ethanol instead of sample to checkup the validity of test.

GGT determination

The GGT level was determined by colorimetric method using assay kit from Linear Company.

Results and Discussion

The results showed a positive test for all beverages except barbican meaning that all the beverages contain alcohol, while barbican does not contain. The positive results means that the color of filter paper test solution is converted to blue color, the density of color is directly proportional to the concentration of alcohol present, so dark blue color means the presence of large percentage of ethanol. The samples of sputum gave a positive result for drunks and negative results for non alcoholics (control). In the sample of low ethanol concentration the color of dichromate disappeared only, this means a positive result. The results are depicted in Table 1 and 2.

The results of GGT determination showed an increase in the level of GGT for drunks in comparison with non alcoholics (control). The results of GGT determination showed an increase in the level of GGT for drunks in comparison with non alcoholics (control) and with normal values of W.H.O. This increase confirm the dichromate test because GGT level is an indicator for alcoholics and its level increases directly with the amount of alcohol consumption.

Table 1: The results of sputum samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Drunks result</th>
<th>Control result</th>
<th>Positive result type</th>
<th>Negative result type</th>
</tr>
</thead>
<tbody>
<tr>
<td>sputum</td>
<td>Positive</td>
<td>Negative</td>
<td>Color changes to blue</td>
<td>No changes in color</td>
</tr>
</tbody>
</table>
Table 2: The results of beverage samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Result</th>
<th>Result type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whisky</td>
<td>Positive</td>
<td>Color change to blue</td>
</tr>
<tr>
<td>vodka</td>
<td>Positive</td>
<td>Color change to blue</td>
</tr>
<tr>
<td>Wine</td>
<td>Positive</td>
<td>Color change to dark blue</td>
</tr>
<tr>
<td>beer</td>
<td>Positive</td>
<td>Color change to light blue</td>
</tr>
<tr>
<td>barbican</td>
<td>Negative</td>
<td>No change in color</td>
</tr>
<tr>
<td>Water</td>
<td>Negative</td>
<td>No change in color</td>
</tr>
</tbody>
</table>

The results depend on the presence of ethanol in the sample; if the sample contains ethanol positive result will appear and negative result appears if the sample does not contain alcohol. The density of appeared color depends up on the concentration of ethanol, dark blue-green color appears in high ethanol concentration and light blue in low concentration and the color of dichromate may disappear only in very small amounts of ethanol. The results are depicted in figure 1. The mechanism of this experiment depends on the presence of alcohol which reacts with an orange-yellow chemical compound called potassium dichromate (K$_2$Cr$_2$O$_7$). When the alcohol reacts with the potassium dichromate, a blue-green compound called Chromium (III) sulfate is produced. The reaction is as follows:

$$2\text{K}_2\text{Cr}_2\text{O}_7 + 8\text{H}_2\text{SO}_4 + 3\text{CH}_3\text{CH}_2\text{OH} \rightarrow 2\text{Cr}_2(\text{SO}_4)_3 + 2\text{K}_2\text{SO}_4 + 3\text{CH}_3\text{COOH} + 11\text{H}_2\text{O}$$

**Conclusion**

The results obtained showed a good method can be used to detect the drunkenness and presence of ethanol in various beverages; this is benefit for the investigation in crime scenes and for qualitative control and measuring. The method is rapid needs for five minutes only.

**Conflict of Interest:** Nil.

**Source of Funding:** Self.

**Ethical Clearance:**

**References**

11. Webmd.com. [Internet]. Peptic Ulcer Disease (Stomach Ulcers) Cause, Symptoms, Treatments. 27 April 2013.
Nurses’ Knowledge Regarding Prevention of Surgical Site Infections at Baghdad Cardiac Centers and Hospitals.

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1Assistant instructor, 2Instructor, 3Instructor, Fundamentals of Nursing Department, College of Nursing-University of Baghdad

Abstract

Background: Surgical site infections (SSIs), are defined as infections that occur within 30 days of the operation. Nosocomial infection, also called hospital acquired infection occur in patient who are admitted in Hospital for a long or short period of time which leading to cause of morbidity and death, therefore, we decided to establish the extent of knowledge of Surgical site infections Prevention among nurses at Baghdad Cardiac Centers and Hospitals. Objectives: the study aimed to assess the nurse’s knowledge regarding prevention of surgical site infections and to find out the relationship between Nurses’ Knowledge and some of demographic data (age, gender, level of education, years of experiences, and training session).

Methods: Across- sectional study was conducted in the period of December 16th, 2018 up to the end of 18th June, 2019. A purposive sample consisted of (50) nurses were selected out of two cardiac centers and one cardiac hospital in Baghdad city. The data collected was analyzed by using SPSS version 20.0 the study instrument consist of two major sections were constructed it is based on the review of literatures. First one is concerned with demographic data for sample, and the second section is knowledge, The extent of knowledge concerning SSI was determined using multiple-choice questionnaires covering the nurse’s knowledge, Question tools is composed of (23) items, reliability and validity of the questionnaire was determined through the analysis of pilot study. Data were collected through the use of self-report method, which analyzed through two statistical approaches. They are descriptive statistical analysis (frequencies, percentage) and inferential statistical analysis (Chi-Square).

Results: the findings of study reveals the study sample reveals that most of the sample (78%) were in age group (25-35) years and most them (52%) were female, the majority of the sample (68%) were married and most of them (42%) were nursing college graduate, the high percent (90%) of them having participation in training session, the majority of their nursing experience in general were (42%) from (1-5) years and most of them (66%) have experience in cardiac care unit from (1-5) years. Conclusions: most of the study sample has moderate knowledge regarding prevention of surgical site infection in Baghdad cardiac centers and hospitals. Recommendation: on the basis of the results of the study the researcher recommends that further research needed with large sample, to find out more result relationships and in different area. And special training programs should be designed and constructed for nurses in these areas to increase their skills.

Keywords: Nursing knowledge, Surgical Site Infections.

Introduction

Surgical site infections are defined as infections that occur within 30 days of the operation (1). One of the most common types of Healthcare-Associated infection is Surgical Site Infection (SSI) which is considered 20% to 25% of all Healthcare-Associated infections (2). (SSI) is an infection that happens among thirty days when an operation causes redness, fever, pain and swelling. It’s additionally one among the foremost imperative complications of a surgical intervention (3) Nosocomial infection have a great impact on the health of hundreds of millions people and it is considered a major global issue today by all the stakeholders (4). Throughout the literature, SSIs were associated with factors including advanced age, malnutrition, metabolic diseases, smoking, obesity, hypoxia, and immune-suppression (5). Nurse’s
pre and post-operative care play a significant influence in infection management responsibilities. Nurses have the distinctive opportunity to reduce the potential for hospital-acquired infections. By utilizing the skills and knowledge of nursing practice, they can facilitate patient recovery while minimizing complications related to infections. Nurses require being knowledgeable regarding the cause, effect and management of SSI to ensure optimum patient’s outcomes following surgery.

Materials and Method

Across-sectional study in which approach is applied in order to achieve the earlier stated objective. The study was initiated from December 16th, 2018 to 18th June, 2019.

A purposive (non-probability) sample of 50 nurses works in cardiac units. The study has been conducted on the Iraqi Center of Heart Disease, Ibn al-Nafees Hospital and Ibn-Albettar Center. Self-administrative questionnaire was modified by investigators for the present study depending on: Review of available literature, the related previous studies scales. The modified questionnaire consist of two parts: the first contained the demographic Characteristics of nursing student consist of (7) items; the second part include nurses’ knowledge Regarding Prevention of Cardiac Surgical Site Infections at Baghdad Cardiac Centers and Hospitals which consist of (23) items rated to tow levels of likers scale and scored the follows: (1) for right answer (0) for wrong answer. Validity for the instrument was determined through the use of panel experts to investigate the clarity, relevancy, and adequacy of the questionnaire in order to achieve the present studies objectives. A pilot study was carried out for period from January 28th, 2019 to February 10th, 2019 and conducted on 10 nurses. Statistical analysis were conducted by using statistical package for social science (SPSS) version 20.0 data analysis was employed through the application of descriptive and inferential statistical approaches.

Results

Table (1): Nurse’s Knowledge of the Study Sample.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency (F)</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>poor</td>
<td>3</td>
<td>6.0</td>
</tr>
<tr>
<td>moderate</td>
<td>25</td>
<td>50.0</td>
</tr>
<tr>
<td>good</td>
<td>22</td>
<td>44.0</td>
</tr>
<tr>
<td>total</td>
<td>50</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table (1): this table presents the nurse’s knowledge regarding Prevention of Surgical Site Infections in Cardiac Centers and hospitals. The table Show that the majority of the sample (50%) were rated as moderate knowledge while (6%) of them have poor knowledge.
Table (2): The Relationship between Nurse’s Knowledge and Hospitals name.

<table>
<thead>
<tr>
<th>Hospital name</th>
<th>Nurse’s knowledge</th>
<th>Total</th>
<th>Chi-square test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Poor</td>
<td>moderate</td>
<td>Good</td>
</tr>
<tr>
<td>Iraqi Center of Heart Disease (F)</td>
<td>2</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>% Within Nurse’s Knowledge</td>
<td>66.7%</td>
<td>24.0%</td>
<td>36.4%</td>
</tr>
<tr>
<td>Ibn al-Nafees Hospital</td>
<td>0</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>% Within Nurse’s Knowledge</td>
<td>0.0%</td>
<td>32.0%</td>
<td>45.5%</td>
</tr>
<tr>
<td>Ibn-Albettar Center</td>
<td>1</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>% Within Nurse’s Knowledge</td>
<td>33.3%</td>
<td>44.0%</td>
<td>18.2%</td>
</tr>
<tr>
<td>Total (F)</td>
<td>3</td>
<td>25</td>
<td>22</td>
</tr>
<tr>
<td>% Within Nurse’s Knowledge</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Where d.f = degree of freedom, sig=significant, N.S = non-significant, F=frequency.

Table (2): this table shows that there are no significant association between nurse’s knowledge and hospitals which they working there. Ibn Al-Nafees Hospital is the high percentage (36 %) of good knowledge for their staff in cardiac units.
Table (3): The Relationship between Nurse’s Knowledge and Years of Experience in Nursing in General.

<table>
<thead>
<tr>
<th>Years of experience in nursing in general</th>
<th>Nurses knowledge</th>
<th>Total</th>
<th>Chi-squares test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Poor</td>
<td>moderate</td>
<td>Good</td>
</tr>
<tr>
<td>1-5 years (F)</td>
<td>0</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>% within nurses knowledge</td>
<td>0.0%</td>
<td>44.0%</td>
<td>45.5%</td>
</tr>
<tr>
<td>6-10 years (F)</td>
<td>1</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>% within nurses knowledge</td>
<td>33.3%</td>
<td>32.0%</td>
<td>27.3%</td>
</tr>
<tr>
<td>11-15 years (F)</td>
<td>0</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>% within nurses knowledge</td>
<td>0.0%</td>
<td>12.0%</td>
<td>22.7%</td>
</tr>
<tr>
<td>16-20 years (F)</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>% within nurses knowledge</td>
<td>33.3%</td>
<td>0.0%</td>
<td>4.5%</td>
</tr>
<tr>
<td>21-25 years (F)</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>% within nurses knowledge</td>
<td>33.3%</td>
<td>4.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>26-30 years (F)</td>
<td>0.0%</td>
<td>8.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>% within nurses knowledge</td>
<td>0.0%</td>
<td>4.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Total (F)</td>
<td>3</td>
<td>25</td>
<td>22</td>
</tr>
<tr>
<td>% within nurses knowledge</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Where d.f = degree of freedom, sig=significant, N.S =non-significant, F=frequency.

**Discussion of the Nurse’s Demographic Characteristics**

Through the data Analysis of the present study the findings shows that most of the study samples (78%) were in age group (25-35) years and most them (52%) were female.
This result agree with the findings obtained from other study, which shows that the majority (73%) of the studied nurses had 21 years and less than 30 years and most of them (52%) were female(9).

Regarding the marital status of our study the results shows that the majority of the samples (68%) were married and most of them (42%) were nursing college graduate.

This result is agree with findings obtained from others study which found B.S (89% ) are more likely to have better knowledge than diploma holders(10).

This agrees with result obtained from other study which found that the highest percentages (40.0%) of the sample were academic nurses who graduated from Colleges of Nursing(11).

According to training session the results shows that Most of our study sample (90%) having participation in training session, this study disagree with another study which found that More than half of studied nurses said that they; did not have any previous training course(12).

The results of our study shows that the majority of nursing experience in general were (42%) from (1-5) years and most of them (66%) have experience in cardiac care unit from (1-5) years.

This result agree with another study which found that Most of the study have work experience (52%) of sample from (1-5) years(13).

Discussion of Nurses Knowledge Regarding Prevention of Surgical Site Infections in Cardiac Centers and Hospitals.

In this study the results reveal that the nurses have a good knowledge about (sign and symptom of SSI?), (intraoperative prevention of SSI), (prophylactic prevention of SSI?), (postoperative prevention of SSI), (form discharge instruction?), (what is the purpose for pre-operative skin preparation), (which one is the correct answer for the benefit of wound dressing?). While about (treatment of SSI?), (when is the best time for pre-operative hair remove?), (which one is best agent for preoperative skin preparation?), (when should you administer prophylaxis antibiotic surgical patient), the nurses have deficit knowledge, related prevention of Surgical Site Infections in Cardiac Centers and hospitals.

The results disagree with findings of another study which found that the majority of the samples (32%) have good knowledge regarding infection control(14).

Discussion of the Nurse’s Knowledge of the Study Sample.

The Results of this study Shows that the majority of the sample (50%) were rated as moderate knowledge while (6%) of them have poor knowledge.

These results agree with the previous study that found the proportion of nurses who were knowledgeable about prevention of surgical site infection was found to be 40.7% with a mean score of 56.3%. This finding indicated that more than half of the nurses demonstrated inadequate knowledge on prevention of surgical site infections(15).

Discussion of Relationship between Nurse’s Knowledge and Hospitals which working there.

The result of our study shows that there are no significant association between nurse’s knowledge and hospitals which working there. Hospital No. (2) Ibn Al-Nafees Hospital is the high percentage (36 %) of good knowledge for their staff in cardiac units.

Discussion of the Nurse’s Knowledge and Years of Experience in Nursing in General.

The result of the present study report that there are significant differences between nurse’s knowledge and Years of Experience in nursing in General.

This finding is in line with findings from European and African studies in which found that the years of experience was positively associated with knowledge regarding infection prevention(16).

Conclusion

Most of the study samples have moderate knowledge regarding prevention of surgical site infection in Baghdad cardiac centers and hospitals.

Recommendation: on the basis of the results of the study the researcher recommends that further research needed with large sample, to find out more result relationships and in different area. And special training programs should be designed and constructed for nurses in these areas to increase their skills.
Acknowledgement: This research was funded by Author. Moreover we would like to thank the study participants and data collectors for their fully participation and responsible data collection.

Funding: None.

Conflict of Interest: None declared.

Ethical approval: Not required.

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2. Owens, C., Stoessel, K.; Surgical site infections: epidemiology, microbiology and prevention. Journal of Hospital Infection, 2008; 70: 3-10]
11. Al-Ganmi A. Assessment of Nurses’ Knowledge Concerning Cardiogenic Shock for Patients’ in Cardiac Care Unit at Baghdad Hospitals. kufo Journal for Nursing sciences. 4.2 (2014): 215-222]
Molecular Docking, Pharmacokinetics, and Toxicity Prediction of Epigallocatechin-3-Gallate (EGCG) on IKK Receptor in Photoaging Prevention

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Abstract

Photoaging is skin aging, caused by chronic exposure of ultraviolet radiation. Photoaging decreases patients’ quality of life because the skin was the outer organ seen by others. Ultraviolet radiation causes oxidative stress, that activated inhibitory kappa B kinase (IKK), increased nuclear factor kappa B (NF-kB), matrix metalloproteinase (MMP), and degradation of collagen. Epigallocatechin-3-gallate (EGCG) was the main green tea polyphenol and the main source of biologic activity of green tea. This study was an in silico study, aimed to obtain the effectiveness of EGCG component through molecular docking on IKK receptor (PDB ID: 5EBZ). The bioinformatics tools based on reverse docking used in this study, were Protein Data Bank, ChemDraw, Chem3D, and Molegro Virtual Docker software. Docking and binding site analysis showed, that EGCG was able to interact with IKK receptor. Rerank score of interaction between EGCG and IKK receptor was higher than that of arbutin and 5TL_701[A]. It showed that EGCG has higher potential in photoaging prevention than arbutin, as one of the agents in photoaging prevention. Pharmacokinetics and toxicity (ADMET) prediction in this in silico study were conducted using pkCSM On Line tool. The pkCSM results showed EGCG was predicted having good pharmacokinetics profile without toxicity effect.

Keywords: photoaging, EGCG, IKK, docking, pharmacokinetics, toxicity, ADMET.

Introduction

Photoaging is extrinsic skin aging, caused by ultraviolet radiation. Photoaging plays role in 80-90% of skin aging procession. Geriatric population in developed and developing countries were increased. Geriatric population in the United States in 2000 was 13% (35 million people), and predicted it would be 30% in 2030. Increasing life expectancy will increase the aging problems, especially skin aging because the skin is the outer layer of human organ seen by others. Photoaging decreases patients’ self-esteem and quality of life.1,5

Inhibitory kappa B kinase (IKK) plays an important role in photoaging pathogenesis, and are an attractive target for photoaging prevention. The IKK receptor can be used as a target in photoaging prevention and can be applied in molecular modeling and structure-activity relationship based drug design.6,7,8,9 The biggest problems in photoaging prevention are drug effectivity and drug efficiency. The discovery of new drugs with the effective target, that was started with research on drug design, is needed in photoaging prevention.10

The drug discovery and development are complicated processions. These processions need a long duration of time and expensive cost. The methods of drug discovery and development are divided into 2 methods, high throughput screening (HTS) dan virtual
screening. High throughput screening (HTS) consist of chemical compound synthesis and screening based on the protein. Synthesis of a chemical compound, in vitro study, and low hit rate were performed in all pharmacy company, but the high cost in these processions are always the biggest problem.\textsuperscript{(11,12)}

Drug discovery and development term become drug design, so the knowledge about biochemical procession and protein that play role in the pathogenesis of diseases, and drug design in modulating this protein are needed. In silico study, in vitro study, and in vivo study are completing each other in drug design procession.\textsuperscript{(11,13)}

In vitro study of EGCG for photoaging prevention has already done. It can prevent photoaging by inhibiting cJun terminal kinase (JNK) and p38-mitogen-activated protein kinase (p38 MAPK) pathway.\textsuperscript{(14)} This study was an in silico study, aimed to obtain the effectiveness of EGCG component through molecular docking on IKK receptor (PDB ID: 5EBZ).

In silico study in drug development is based on protein-drug interaction, by docking procession. The reactivity of protein is based on protein structure and chemical bond (hydrogen bond, van der Waals bond, covalent bond, and ionic bond). Computer-aided drug delivery (CADD) is able to show computational analysis of protein reactivity, by evaluating protein structure, chemical bond, and protein-drug interaction.\textsuperscript{(15)}

\textbf{Material and Method}

The molecular structure of IKK receptor was downloaded from protein data bank (PDB), and PDB ID: 5EBZ was selected. The structure of ligands was drawn using ChemDraw software application, version 11 and copied into Chem 3D software application, version 11 to create the 3D structure and measure its minimum energy using Molegro Virtual Docker, version 5.5. The validation of the docking study was performed by re-docking the ligand reference into an appropriate protein cavity. Re-docking is accepted if the root mean square value (RMSD) $< 2.0 \text{ Å}$.\textsuperscript{(16)}

The docking study of EGCG on the IKK receptor (PDB ID: 5EBZ) was conducted using Molegro Virtual Docker, version 5.0 (processor: Intel (R) Pentium (R) CPU N4200 @1.10GHz; installed RAM: 4.00 GB; system type: 64-bit operating system). The best docking results were detected visually by comparing the structure of the docked molecules with the structure of reference ligand (STL\_701[A] or 6'-amino-5'-(amino(hydroxy)methyl)-1,2,3,6'-tetrahydro-[1,1':3',1''-terpenyl]-4-sulfonamide) in the binding site. The MolDock and ReRank scores have presented the energy needed in receptor-ligand bond (Table 1). The lowest energy visualized the best binding pose between the ligand and amino acid residue of the protein.\textsuperscript{(16)}

\begin{table}
\centering
\begin{tabular}{|c|c|}
\hline
Ligand & MolDock Score ReRank Score \\
\hline
EGCG & -5.3 -5.5 \\
Arbutin & -5.1 -5.3 \\
Reference & -5.5 -5.7 \\
\hline
\end{tabular}
\caption{MolDock and ReRank scores of EGCG, arbutin, and reference ligand in IKK cavity.}
\end{table}

Pharmacokinetics prediction (absorption, distribution, metabolism, excretion) and toxicity prediction of EGCG, arbutin, and reference ligand were performed using pkCSM On-Line Tool. The molecular structure of EGCG, arbutin, and reference ligand were drawn as 2D molecular structures with ChemDraw software, copied into Chem3D software to create 3D structure, and stored as a .sdf file. The .sdf format of EGCG, arbutin, and reference ligand were translated into SMILE format using SMILE Translator Online Help. The SMILE format was processed using the pkCSM Online Tool to predict the pharmacokinetics and toxicity of compounds.\textsuperscript{(16,17,18,19)}

\section*{Findings}

Molecular docking was performed to evaluate the mode of binding between the compound and IKK receptor (PDB ID: 5EBZ). The result of molecular docking 3D structure between candidate ligand (EGCG), control ligand (arbutin), and reference ligand (STL\_701[A]) in IKK cavity showed, that the ligands were able to interact with IKK receptor as the target protein (PDB ID: 5EBZ) on the same binding site (Figure 1).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{Figure1.png}
\caption{The result of molecular docking 3D structure between candidate ligand (EGCG), control ligand (arbutin), and reference ligand (STL\_701[A]) in IKK cavity. Description: green (IKK cavity), red (EGCG), yellow (arbutin), white (STL\_701[A]).}
\end{figure}
The best docking position in the 3D structure molecules of EGCG to IKK receptor (PDB ID: 5EBZ) can be seen in Figure 2A. The docking was carried out at cavity 5, vol. 86.016; surface: 296.96. The bond location of the ligand binding site and target protein showed, that EGCG interacted with IKK receptor through 54 number of bonds. Hydrogen and steric bond from 10 amino acids (Asp 165, Asn 149, Ile 150, Val 74, Leu 166, Met 65, Ile 164, Met 95, Val 29, and Cys 98) were showed at Figure 2B. The Mol Dock score and Rerank score of interaction between EGCG and 5EBZ in IKK cavity were shown in Table 1.

**Table 1: Moldock Score And Rerank Score Of Interaction Between 5EBZ Protein And Compounds**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>MolDock Score (kcal/mol)</th>
<th>Rerank Score (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGCG</td>
<td>-154.7±7.80</td>
<td>-115.8±2.96</td>
</tr>
<tr>
<td>Arbutin</td>
<td>-84.52±0.03</td>
<td>-79.05±0.85</td>
</tr>
<tr>
<td>5TL_701[A] as ligand</td>
<td>-128.31±2.24</td>
<td>-84.46±0.75</td>
</tr>
</tbody>
</table>

The best docking position in the 3D structure molecules of EGCG in IKK cavity using Molegro Virtual Docker software. EGCG (red) was bound to target protein (5EBZ). (B) Hydrogen and steric bound between EGCG and target protein (5EBZ).

Figure 3 (A) Hydrophobicity view of the interaction between arbutin in IKK cavity using Molegro Virtual Docker software.
Arbutin (yellow) was bound to target protein (5EBZ). (B) Hydrogen and steric bound between arbutin and target protein (5EBZ).

Arbutin is a topical agent that is used in photoaging prevention. The best docking position in the 3D structure molecules of arbutin to IKK receptor (PDB ID: 5EBZ) can be seen in Figure 3A. The docking was carried out at 5, vol. 86.016; surface: 296.96. The bond location of the ligand binding site and target protein showed, that control ligand (arbutin) interacted with IKK receptor through 36 number of bonds. Hydrogen and steric bonds from 7 amino acids (Cys 98, Gly 22, Gly 24, Thr 23, Val 29, Asp 165, and Leu 21) were showed in Figure 3B. The Mol Dock score and Rerank score of interaction between arbutin and 5EBZ in IKK cavity were shown in Table 1.

The result of molecular docking between IKK receptor (PDB ID: 5EBZ) with a candidate ligand (EGCG), a control ligand (arbutin, as one of topical agent in photoaging prevention), and a reference ligand (5TL_701[A]) using Molegro Virtual Docker software, showed that the binding affinity of IKK receptor with EGCG to be higher than that of arbutin. Based on Table 1, the average Mol Dock and Rerank score of interaction between IKK receptor and EGCG were -154.7±7.80 kcal/mol and -115.8±2.96 kcal/mol; between IKK receptor and arbutin were -84.52±0.03 kcal/mol and -79.05±0.85 kcal/mol; and between IKK receptor and 5TL_701[A] were -128.31±2.24 kcal/mol and -84.46±0.75 kcal/mol.

Based on the in silico study of the physicochemical properties of EGCG, the molecular weight value was 458.375 (<500), and the value of the log of octanol/water partition coefficient (log P) was 2.2332. The result of pharmacokinetic prediction of EGCG can be seen in Table 2.

The absorption of a compound is very important because it determines the action of the compound. The pkCSM intestinal absorption prediction is based on the proportion of compounds that were absorbed via the human small intestine. If the pkCSM intestinal absorption prediction value is less than 30%, it is considered to be poorly absorbed. The absorption of EGCG was 48%, higher than arbutin. Therefore, it can be predicted that EGCG has moderate intestinal absorption.

Table 2: Pharmacokinetics properties of EGCG, arbutin, and 5TL_701[A]

<table>
<thead>
<tr>
<th>Pharmacokinetics properties</th>
<th>Model name</th>
<th>Predicted value (EGCG)</th>
<th>Predicted value (Arbutin)</th>
<th>Predicted value (5TL_701[A])</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorption</td>
<td>Intestinal absorption (human)</td>
<td>48.191</td>
<td>42.175</td>
<td>73.943</td>
<td>% Absorbed (Numeric)</td>
</tr>
<tr>
<td></td>
<td>Skin Permeability</td>
<td>-2.735</td>
<td>-2.743</td>
<td>-2.737</td>
<td>Log Kp (Numeric)</td>
</tr>
<tr>
<td>Distribution</td>
<td>BBB permeability</td>
<td>-2.091</td>
<td>-0.865</td>
<td>-0.924</td>
<td>Log BB (Numeric)</td>
</tr>
</tbody>
</table>
**Metabolism**

<table>
<thead>
<tr>
<th>Protein/Substrate</th>
<th>No</th>
<th>No</th>
<th>No</th>
<th>Yes/No (Categorical)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6 substrate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP3A4 substrate</td>
<td></td>
<td></td>
<td>Yes</td>
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</tr>
<tr>
<td>CYP1A2 inhibitor</td>
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<td>CYP2C19 inhibitor</td>
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<td>CYP2D6 inhibitor</td>
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<td>CYP3A4 inhibitor</td>
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**Excretion**

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<th>Property</th>
<th>Value</th>
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<td>Total Clearance</td>
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<td></td>
<td>0.595</td>
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**Excretion**

<table>
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<th>Value</th>
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<tbody>
<tr>
<td>Log ml/min/kg</td>
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**Toxicity**

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<th>Value</th>
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<tbody>
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<td>AMES toxicity</td>
<td>No</td>
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<tr>
<td>Hepato-toxicity</td>
<td>No</td>
</tr>
<tr>
<td>Skin Sensitisation</td>
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</table>

**Discussion**

The potentially interactive target protein with EGCG was IKK receptor. The IKK receptor plays role in inhibition of kappa B kinase, which activated NFkB. Nuclear factor kappa B (NFkB) plays role in photoaging pathogenesis, by activating matrix metalloproteinase (MMP) and increasing collagen degradation.\(^{5,19,20}\) It was predicted, that inhibition of IKK receptor by EGCG would be able to prevent photoaging.

The binding affinity of EGCG to IKK receptor was higher than that of arbutin and reference ligand (5TL_701[A]), and it showed that EGCG has higher potential than arbutin and reference ligand (5TL_701[A]) to be an alternative agent in photoaging prevention.

The blood-brain barrier (BBB) protects the brain from the exogenous compound. If the logBB is more than 0.3, it is considered that the compound is able to across blood-brain barrier and enter to the brain, while the logBB less than -1 showed that the compound is poorly across blood-brain barrier and enter to the brain.\(^{17,18}\) The BBB permeability of EGCG was -2.091 (Table 2), lower than arbutin and reference ligand. Therefore, it can be predicted that EGCG is poorly distributed to the brain. It can also be predicted EGCG gives minimal side effect and toxicity into the brain.

The most important detoxification enzyme in the body in liver is cytochrome P450. Cytochrome P450 deactivated some drugs, and it can also activated several drugs. The drug metabolism are mainly regulated by two isoforms (CYP2D6 and CYP3A4 substrates). These two main isoforms will predict whether a molecule can be metabolized by cytochrome P450. Inhibition of cytochrome P450 may disturb the drug metabolism. The different isoforms of cytochrome P450 (CYP1A2/ CYP2C19/ CYP2C9/ CYP2D6/ CYP3A4) were built.
These different isoforms were able to inhibit cytochrome P450. The predictors in pkCSM can predict whether a molecule was an inhibitor of cytochrome P450 or whether a molecule metabolised by cytochrome P450. Table 2 showed that EGCG is not likely to be metabolized by cytochrome P450 and does not inhibit CYP1A2, CYP2C19, CYP2C9, CYP2D6, but inhibits CYP3A4. Therefore, it can be predicted that EGCG is unable to metabolize by cytochrome P450 and EGCG is not likely going to be a cytochrome P450 inhibitor.

Total clearance of the drug is measured by proportionally constant CL tot, and it is a combination of hepatic and kidney clearance. Total clearance is related to the bioavailability of a molecule. The total clearance predictors is given in log(ml/min/kg). Table 2 showed that the prediction of total clearance of EGCG was 0.406 log ml/min/kg.

Toxicity of compound can be predicted from AMES toxicity, hepatotoxicity, and skin sensitization. The mutagenic potential of the compounds can be predicted from the AMES test. A positive AMES toxicity test indicates that a compound is mutagenic and may become a carcinogen agent. The important safety consideration for new drug development is drug-induced liver injury. The drug-induced liver injury may also cause drug attrition. The hepatotoxicity predictors in pkCSM may predict whether a molecule may disturb the function of the liver. The most potential adverse effect from topical drug application is skin sensitization. The most important safety consideration of topical drug is the evaluation of whether a compound can induce allergic contact dermatitis. It was predicted from pkCSM that EGCG does not induce mutagenic effect, hepatotoxicity, and skin sensitization.

Conclusion

This in silico study showed, that EGCG has potential in photoaging prevention, by interacting with IKK receptor (PDB ID: 5EBZ). EGCG was predicted having good pharmacokinetics profile and no toxicity effect to be an alternative agent in photoaging prevention.

Conflict of Interest: No conflict of interest regarding the publication.

Source of Funding: This research was financially supported by Directorate of Research and Community Service - Directorate General of Research and Development - Ministry of Research, Technology and Higher Education (Direktorat Riset dan Pengabdian Masyarakat - Direktorat Jenderal Riset dan Pengembangan - Kementerian Riset, Teknologi dan Pendidikan Tinggi/Kemenristekdikti) Indonesia.

Ethical Clearance: Taken from Ethical Committee in Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia.

References


Correlation between High Serum Uric Acid Levels with Occurrence of Diabetic Peripheral Neuropathy in Patients with Type 2 Diabetes

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Abstract

Background: Diabetic Peripheral Neuropathy is a chronic microvascular complication of type 2 diabetes mellitus (T2DM) leads to increased risk of foot ulceration and morbidity. The increased serum uric acid (SUA) levels have been linked to macro vascular disease in T2DM. We found that the correlation between SUA levels and diabetic peripheral neuropathy has not been investigated.

Objective: To determine the correlation between high serum uric acid levels and diabetic peripheral neuropathy.

Method: The case-control design was used in this study and the sampling was done consecutively by following the inclusion and the exclusion criteria. The diabetic peripheral neuropathy was evaluated using EMNG and the serum were taken for uric acid levels examination. Chi square test was used for data analysis.

Results: Thirty subjects were enrolled and divided into an experimental group of 15 subjects and a control group of 15 subjects as well. We found that the diabetic peripheral neuropathy did not show a significant correlation with high serum uric acid levels, p=0.136 and OR 3.143 (CI 95% 0.681-14.503).

Conclusion: High serum uric acid levels did not have correlation with diabetic peripheral neuropathy.

Keywords: High Serum, Neuropathy, Diabetes Mellitus

Introduction

Diabetes mellitus (DM) is a clinical syndrome characterized by hyperglycemia that occurs due to insulin secretion abnormalities, insulin performance, or both. Type 2 Diabetes Mellitus (T2DM) is an increasing health problem of incidence and prevalence so it becomes a worldwide concern. Diabetic Peripheral Neuropathy (DPN) is one of the most frequent chronic microvascular complications in T2DM. Finger or foot infections and amputations are common risks faced by DPN. This causes an increase in morbidity and mortality resulting in increased medical costs of patients with DPN. Prevalence of neuropathy in DM patients over 50% for 25 years. The overall prevalence of neuropathy was estimated at 30%. In the EURODIAB IDDM Complication Study, DPN is associated with blood glucose control and DM duration. Microvascular complications still occur, despite controlling of blood glucose levels was performed well (HbA1c 5.4% to 7%), so it is suspected other factors involved besides blood glucose control and duration of DM.

High serum uric acid (SUA) levels were associated with the incidence of macrovascular and microvascular complications in patients with DM. Increased levels of SUA have been associated with endothelial dysfunction, ischemic heart disease, stroke, peripheral artery disease and death from cardiovascular disease. In T2DM, the increased levels of SUA were associated with metabolic syndrome and insulin resistance. The association of high SUA levels with DM was reported in several studies. The association of hyperuricemia with DPN is still controversial. The study by Ito et al (2011)
shows that DPN had a moderate significance (p < 0.001) with a positive correlation (r=0.509) towards high SUA levels. Therefore, the purpose of this study was to determine the correlation between high serum uric acid levels and diabetic peripheral neuropathy.

**Method**

This study used a case control design with the population of all T2DM patients who visited to the endocrinology outpatient unit of Soetomo General Hospital and fulfilled the inclusion and the exclusion criteria during the period of August to December 2016. The inclusion criteria were 40-60 years old, EMNG results did not support the DPN and agreed to follow the research. While the exclusion criteria were chronic renal impairment, chronic liver disorder, history of malignancy, history of drug use and radiotherapy, history of alcohol consumption.

We used consecutive sampling as the sampling technique because it was the best non-probability sampling and easy to do. The sample size was determined using the formula of unpaired categorical analytic research. The value of the effect proportion on the control (P2) was determined based on the preliminary study and the calculation of the required minimum sample size was 15 people for each group.

The samples were divided into two groups; 15 subjects with EMNG results that did not support the diabetic neuropathy referred to as control group and 15 subjects with EMNG results that supported the diabetic neuropathy, hereinafter referred to as case/experimental group. The sampling conducted for 5 (five) months.

Additionally, the T2DM patients who fulfilled the inclusion and the exclusion criteria will be performed anamnesis, physical examination and then performed the blood tests for serum uric acid levels and other confounding factors. Statistical analysis was performed using SPSS 20.0 program. Collected categorical data were analyzed using Chi-Square test while numerical data were analyzed by T-test.

**Result**

The total subjects were 30 patients consisting of 15 subjects with EMNG results that did not support the DPN that referred to as control group and 15 subjects with EMNG results that supported the DPN, hereinafter referred to as case/experimental group. The basic characteristics of research subjects consisting of demographic and clinical data were showed in table 1. While the correlation between high serum uric acid levels and the incidence of diabetic peripheral neuropathy was shown in table 2.

**Table 1. The Basic Characteristics of Subjects**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Experimental</th>
<th>DPN Incidence</th>
<th>OR</th>
<th>p</th>
<th>(CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Male</td>
<td>5 (33.3 %)</td>
<td>5 (33.3 % )</td>
<td>10 (33.3 %)</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>- Female</td>
<td>10 (66.7 %)</td>
<td>10 (66.7 %)</td>
<td>20 (66.7 %)</td>
<td>(0.219-4.564)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>51.60±6.52</td>
<td>53.53±4.72</td>
<td>0.360</td>
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<td></td>
</tr>
<tr>
<td>Body Mass Index (BMI)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Normal</td>
<td>9 (60.0 %)</td>
<td>9 (60.0 %)</td>
<td>18 (60.0 %)</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>- Obesity</td>
<td>6 (40.0 %)</td>
<td>6 (40.0 %)</td>
<td>12 (40.0 %)</td>
<td>(0.232-4.310)</td>
<td></td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td></td>
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<td></td>
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</table>
**Table 1. The Basic Characteristics of Subjects**

<table>
<thead>
<tr>
<th>Serum Uric Acid Levels</th>
<th>DPN Cases</th>
<th>OR</th>
<th>p*</th>
<th>(CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>8 (53.3%)</td>
<td>4 (26.7%)</td>
<td>12 (40%)</td>
<td>0.136</td>
</tr>
<tr>
<td>Normal</td>
<td>3 (20.0%)</td>
<td>5 (33.3%)</td>
<td>8 (26.7%)</td>
<td>0.681-14.503</td>
</tr>
<tr>
<td>Total</td>
<td>15 (100%)</td>
<td>15 (100%)</td>
<td>30 (100%)</td>
<td>(0.117-2.549)</td>
</tr>
</tbody>
</table>

Table 2. The Correlation between High Serum Uric Acid Levels and the Incidence of Diabetic Peripheral Neuropathy

<table>
<thead>
<tr>
<th>DPN Cases</th>
<th>OR</th>
<th>p*</th>
<th>(CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental</td>
<td>Control</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>8 (53.3%)</td>
<td>4 (26.7%)</td>
<td>12 (40%)</td>
</tr>
<tr>
<td>Normal</td>
<td>7 (46.7%)</td>
<td>11 (73.3%)</td>
<td>18 (60%)</td>
</tr>
<tr>
<td>Total</td>
<td>15 (100%)</td>
<td>15 (100%)</td>
<td>30 (100%)</td>
</tr>
</tbody>
</table>

*) Pearson Chi-Square

**Discussion**

This study shows that there were no significant differences in basic variables consisting of demographic data. In addition we obtained that there were no significant differences in clinical data except the T2DM duration. Demographic data included age and sex while clinical data included BMI, dyslipidemia, hypertension, smoking, serum HbA1c levels and T2DM duration.
The mean age of the experimental group (51.60±6.52 years) was slightly younger than the control group (53.53±4.72 years), but this difference was not statistically significant with \( p=0.360 \). This result was in accordance with a study by Volmer et al that the mean age of samples that has suffering from DPN was 56.9±9.6 years \(^9\). But the mean age in this study was relatively young compared to the research that conducted by Wallace et al that the mean age of samples suffering DPN was 68.27±10.66 and the mean age that did not suffer DPN was 62.26±10.05 \(^10\). Multicenter studies conducted in the United Kingdom reported that the prevalence of diabetic peripheral neuropathy increases with age, from 5% (3.1-6.9%) in the 20-29 years age group to 44.2% (41.1-47.3%) in the 70-79 years age group. However, the samples over the age of 60 were not included in this study, because the age affects other types of neuropathy such as neuropathy due to vitamin deficiency, malnutrition, and others \(^11\).

Characteristics of the subjects based on body mass index (BMI) in the experimental group were the subjects with normal BMI that was 9 patients (60.0%) meanwhile the subject of obesity in 6 patients (40.0%). However, this difference was not statistically significant with \( p=1.000 \). This was in accordance with a study that conducted by Koe et al., reported that there was also no significant difference between BMI and diabetic neuropathy \( p=0.056 \) in 84 subjects. Obesity or combination with metabolic syndrome was a risk factor for neuropathy complications \(^12\). Obesity and triglycerides were associated with the loss of small axon nerves that were unveiled myelin.

Obesity was associated with edema that precedes the occurrence of clamp phenomena that disrupt the barrier so that nutritional deficiencies in susceptible nerve tissue. Obesity along with other metabolic syndromes leads to an increase in insulin resistance. T2DM and obesity have a complex relationship. Obesity was a precursor of T2DM via insulin resistance mechanism \(^13\). Characteristics of the subjects based on the risk factor of dyslipidemia in the experimental group were found in 10 patients (66.7%) that less than the control group as many as 13 patients (86.7%).

Study conducted by Putri et al who found that dyslipidemia in the diabetic neuropathy group was 8% while in the diabetic neuropathy group was 44% with \( p=0.075 \) \( p <0.001 \).\(^24\) The study of dyslipidemia as a risk factor for neuropathy was still controversial. There was no significant difference between total cholesterol, HDL, LDL and triglyceride levels in people with type 2 DM who had somatic neuropathy with or without neuropathy. Patients with type 2 DM who were treated intensively with statins reduce the risk of autonomic neuropathy, but not DPN \(^14\).

The different studies found that dyslipidemia was an independent risk factor for macro vascular disease in type 2 diabetes mellitus patients. In the preliminary study, decreased lipid levels with both fibrates and statins within 5 years prevented the incidence of new sensory neuropathy \(^3\). The decrease in lipid levels was performed using fibrate therapy \( HR = 0.52; 95\% CI 0.27-0.98 \) and the use of statins therapy \( HR = 0.65; 95\% CI 0.46-0.93; \ p<0.042 \).\(^32\) The results was consistent with the in vitro studies and animal studies that showed the levels of lipid-lowering therapy had the neuroprotective effects by improving the Schwann cells, polyol pathway, and the repair of nerve blood flow \(^2\).

The characteristics of subjects based on hypertension in the experimental group found 4 patients (26.7%) with hypertension, then fewer than the control group that was 10 patients (40.0%). But in this study there was no statistically significant difference between the experimental and control group \( p=0.439 \). West et al.’s study also found that there was no significant difference between the history of hypertension \( p=0.124 \), systolic blood pressure \( p=0.373 \), and diastolic blood pressure \( p=0.640 \) with diabetic neuropathy. Hypertension was an independent factor in macro vascular disease, retinopathy, and nephropathy. Hypertension was a complication of blood vessels due to hyperinsulinemia. Insulin resistance increases sodium reabsorption in the proximal tubules of the kidney \(^15\).

The characteristics of subjects based on smoking were 1 person (6.7%) who smoked in the experiment group while in the control group there were 14 people who did not (93.3%). But in this study there was no statistically significant difference between the experiment and control group \( p=1.000 \). Additionally, the characteristics of subjects based on long-term T2DM ≥5 years in the case group was 12 people (80.0%) it was more than the control group that only 2 people (13.3%). While the subjects who suffer from DM <5 years in the case group was 5 people (20.0%), less than the control group that was 13 people (86.7%). The correlation test results showed that there was a correlation between the duration of DMT2 and the incidence of diabetic
peripheral neuropathy (p=0.000; odds ratio 0.038; 95% CI 0.005-0.271). This was consistent with some studies obtained a correlation between the duration of DM and diabetic neuropathy 16. Research conducted by Fatkhur et al stated that the longer the patient suffering from DM, the risk of diabetic neuropathy was 16.7787 times greater in DM ≥5 years (p = 0.000). In addition to hyperglycemia, the duration of DM was a risk factor for DPN in Diabetes Control and Complications Trial.

Moreover, characteristics of the subjects by high serum levels of HbA1c as many as 12 people in the experimental group (80.0%) was more than the control group as many as 10 people (66.7%). Based on the risk factor of serum uric acid level in the group of the incidence of peripheral diabetic neuropathy was obtained 8 people (53.3%) with high serum uric acid level, it was more than in the group without peripheral paralytic neuropathy that found 4 people with high serum uric acid level (26.7%).

However, there was no statistically significant difference with p=0.136 and odds ratio by 3.143 (95% CI 0.681-14.503) in this study. The result of odds ratio (OR) was more than 1, so then it was concluded that high serum uric acid levels had no correlation with the incidence of diabetic peripheral neuropathy in type 2 diabetes mellitus. It was not in accordance with this research that conducted by Rafie et al against 132 patients with T2DM aged 45-80 years who suggested there was a correlation between high serum uric acid and diabetic peripheral neuropathy in DM patients (p <0.0001).

Next, the difference could be caused by the age of research. The subjects in this study was 40-60 years while the subjects by Rafie et al up to 80 years. In general, the older age also affects the incidence of peripheral diabetic neuropathy. The multicenter study conducted in the UK, the prevalence of diabetic peripheral neuropathy increased with age, from 5% (3.1- 6.9%) in the 20-29 year age group to 44.2% (41.1-47.3%) In the 70-79 age group 17. The risk factor that affected the DPN was the duration of T2DM. In the experimental group with T2DM duration ≥5 years more than the control group and statistically significant with p=0.000.

**Conclusion**

There was no correlation between the high serum uric acid levels and the incidence of diabetic peripheral neuropathy in patients with T2DM in Soetomo General Hospital Surabaya in August until December 2016.

**Ethical Clearance:** This research involves participants in the survey using sampling method that was accordant with the ethical research principle based on the regulation of research ethic committee. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, nonmaleficence, and justice.

**Conflict of Interest:** So far there is no conflict involved with this paper.

**Source of Funding:** All of the research related with this paper is done by authors’ fund only.

**References**

9. Volmer-Thole M, Lobmann R. Neuropathy


Nephrotoxicity Effects of Post-Cisplatin Paclitaxel Chemotherapy in Severe Head and Neck Tumor

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Abstract

Background: Cisplatin-paclitaxel is a combination of chemotherapy drugs used in the treatment of head and neck malignancy. The main side effect of cisplatin is nephrotoxicity. Nephrotoxicity manifests in decreased glomerular filtration rate (GFR), increased blood urea nitrogen (BUN) and creatinine serum, and decreased magnesium and potassium serum. Objective: this study aimed to determine the nephrotoxicity effects of post cisplatin-paclitaxel chemotherapy in patients with malignant head and neck tumors.

Method: Patients with malignant head and neck tumor who received cisplatin-paclitaxel chemotherapy were taken consecutively and examined their GFR, BUN, creatinine, magnesium and potassium pre- and post-chemotherapy. The data were statistically analyzed whether nephrotoxicity happened post cisplatin-paclitaxel chemotherapy.

Result: Thirty five samples consisted of 24 men and 11 women. The diagnose were 25 nasopharyngeal carcinoma (71.42%), 3 of each sinonasal carcinoma and laryngeal carcinoma (8.57%), and 1 of each tongue carcinoma, parotid carcinoma, tonsillar carcinoma and MAE carcinoma (2.86%). The average of pre-chemotherapy GFR was 94.43±17.44 ml/min, average of post-chemotherapy GFR was 68.17±17.96. Mostly found decreased mild kidney function in 24 (68.57%) post chemotherapy patients.

Conclusion: There was a nephrotoxicity effects after cisplatin-paclitaxel chemotherapy treatment in patients with head and neck malignant tumors treated

Keywords: Nephrotoxicity, Chemotherapy, Cisplatin-paclitaxel, Malignant head and neck tumor.

Introduction

Malignant head and neck tumors are a major problem in ENT unit. Research in Dr. Soetomo General Hospital 1996 - 2000 by got 2119 patients with malignant head and neck tumors. New patients in 2009 - 2012 that underwent treatment in ENT outpatient unit Dr. Soetomo General Hospital were 1479 patients of 15867 total visits ¹. In the United States, 5% of all malignancies in 2006, was found 500,000 new cases. The majority of malignancies in head and neck (>75%) are squamous cell carcinomas, which are relatively radiosensitive. Approximately one-third still has an early stage and two-thirds are in locally advanced stage at the time of treatment ².

Chemotherapy is one of the alternative options in malignant head and neck tumors advanced. The most commonly chemotherapy used in Lotus inpatient unit Dr. Soetomo General Hospital Surabaya is a combination of cisplatin - paclitaxel (cisplatin based), 31% of 2013 patients who received chemotherapy in 2009-2012. The use of cisplatin alone reached 55% in combination with other regimens in chemotherapy of head and neck malignancy ³.

The nephrotoxicity of cisplatin had been recognized since 25 years ago, but since no other drug had been found to be as effective and less toxic, cisplatin continues to be used. Cisplatin nephrotoxicity in experimental animals...
was first discovered in 1971 showing a histopathologic change in the form of acute tubular necrosis accompanied by azotemia. Acute kidney failure due to cisplatin occurs 14-100% of patients, with varied incidents according to dose. Renal insufficiency was found in 20-30% of patients. Onset of renal insufficiency usually occurred on day 3 to 5 after cisplatin consumption, which was indicated by an increase of creatinine serum and BUN levels. Hypomagnesemia, hypocalcemia, hypophosphatemia and hypokalemia commonly happened, especially after repeated doses of cisplatin characterized by decreased GFR 4.

Cisplatin chemotherapy led to a significant increase of creatinine (44.87%), and an insignificant increase of BUN (8.71%) compared to before treatment. At electrolyte serum examination there was a significant decrease of magnesium (7.18%), potassium (6.44%), phosphate (16.44%) and calcium (5.94%), significant increase of sodium (1.35%) and chloride (3.85%) compared to before treatment 5. Another study in India reported 15 patients (20.5%) with acute renal injury characterized by a significant increase in creatinine serum levels within the first 48 hours after cisplatin chemotherapy 6. A study in the United States comparing nephrotoxicity increased in 11 patients receiving cisplatin-paclitaxel combination chemotherapy with 14 cisplatin-treated patients alone had decreased creatinine clearance in 9 patients (81%) who received cisplatin-paclitaxel combination and only 4 (29%) of 14 patients treated with cisplatin alone 7.

**Method**

The samples were taken by consecutive sampling. The independent variable in this study was cisplatin-paclitaxel and dependent variables were nephrotoxicity effects assessed by GFR, BUN, serum creatinine, magnesium (Mg++), potassium (K+) blood plasma 8.

Administration of cisplatin-paclitaxel is the process of incorporating chemotherapy drugs consisting of cisplatin and paclitaxel into the body. The dose of cisplatin in this study was 100 mg/m² of body surface and paclitaxel dose in this study was 200 mg/m² of body surface. The effect of nephrotoxicity is a condition that indicates renal impairment due to chemicals characterized by decreased GFR, increased BUN and creatinin, and decreased Mg++ and K+ in 10 days' post-cisplatin-paclitaxel chemotherapy 9. The data collected in sheet was arranged in table then analyzed descriptively and analytically. Normal distribution test on the data used Shapiro-Wilk, while the analytical test used t 2 paired samples if the data were ratio scale and normal distribution, if the requirement was not met, it would use Wilcoxon test 10.

**Results**

It was obtained 35 samples with age distribution of 17-25 years (8.57%), 26-35 years (17.14%), 36-45 years (25.71%), 46-55 years (42.86%), 56-65 years (5.72%). Sex distribution obtained 24 males (68.57%) and 11 females (31.43%). Ethnicity distribution obtained Java was 28 (80%), Madura was 5 (14.28%), Batak and Dayak was 1 each (2.86%). The diagnosis obtained were 25 nasopharyngeal carcinoma (71.42%), 3 of each sinonasal carcinoma and laryngeal carcinoma (8.57%), and 1 of each tongue carcinoma, parotid carcinoma, tonsillar carcinoma and MAE carcinoma (2.86%).

GFR examination was done pre-chemotherapy and post-chemotherapy of cisplatin-paclitaxel. The average of pre-chemotherapy GFR was 94.43±17.44 ml/min, average of post-chemotherapy GFR was 68.17±17.96 ml/min (Table 1). Decreased mild kidney function with GFR values range of 56-90 ml/min was 24 (68.57%) patients. Decreased moderate kidney function with GFR value range of 35-55 ml/min was 6 (17.14%) patients. Severe kidney dysfunction patients with GFR <35 ml/min was 2 (5.72%) (Table 2).

The average of pre-chemotherapy BUN level was 9.11±3.27 mg/dl and post-chemotherapy BUN level was 14.94±13.88 mg/dl (Table 3). The average of pre-chemotherapy creatinin serum levels was 0.84±0.15 mg/dl and post-chemotherapy creatinin serum level was 1.20±0.54 mg/dl (Table 3). The average of pre-chemotherapy Mg++ levels was 2.20±0.31 mg/dl, while post chemotherapy was 1.93±0.35 mg/dl (Table 3). Patients with hypomagnesium in post cisplatin-paclitaxel chemotherapy were 8 (22.86%), 26 (74.28%) patients had normal Mg++ levels, and 1 (2.86%) patient had hypermagnesium (Table 4). The average of pre-chemotherapy K+ level was 4.08±0.42 mmol/l, while post chemotherapy was 3.55±0.52 mmol/l (Table 5).
Table 1. The result of GFR examination pre and post cisplatin-paclitaxel chemotherapy.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-chemotherapy</th>
<th>Post-chemotherapy</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>SD</td>
<td>Average</td>
</tr>
<tr>
<td>GFR</td>
<td>94.43</td>
<td>17.44</td>
<td>68.17</td>
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</tbody>
</table>

Table 2. Frequency of renal function decrease post cisplatin-paclitaxel chemotherapy

<table>
<thead>
<tr>
<th>Renal function decrease</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>24</td>
<td>68.57</td>
</tr>
<tr>
<td>Moderate</td>
<td>6</td>
<td>17.14</td>
</tr>
<tr>
<td>Severe</td>
<td>2</td>
<td>5.72</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>91.43</td>
</tr>
</tbody>
</table>

Table 3. Result of Laboratory finding of pre and post cisplatin-paclitaxel chemotherapy

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-chemotherapy</th>
<th>Post-chemotherapy</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>SD</td>
<td>Average</td>
</tr>
<tr>
<td>BUN</td>
<td>0.84</td>
<td>0.15</td>
<td>1.20</td>
</tr>
<tr>
<td>Creatinin Serum</td>
<td>0.84</td>
<td>0.15</td>
<td>1.20</td>
</tr>
<tr>
<td>Mg++</td>
<td>2.20</td>
<td>0.31</td>
<td>1.93</td>
</tr>
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</table>

Table 4. Hypomagnesium frequency pre and post cisplatin-paclitaxel chemotherapy

<table>
<thead>
<tr>
<th>Mg++</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypomagnesium</td>
<td>8</td>
<td>22.86</td>
</tr>
<tr>
<td>Normal</td>
<td>26</td>
<td>74.28</td>
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<tr>
<td>Hypermagnesium</td>
<td>1</td>
<td>2.86</td>
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<tr>
<td>Total</td>
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<td>100.00</td>
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Table 5. Result of K+ pre and post cisplatin-paclitaxel chemotherapy

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre-chemotherapy</th>
<th>Post-chemotherapy</th>
<th>Changes</th>
<th>T</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>SD</td>
<td>Average</td>
<td>SD</td>
<td>Average</td>
</tr>
<tr>
<td>K+</td>
<td>4.08</td>
<td>0.42</td>
<td>3.55</td>
<td>0.52</td>
<td>0.53</td>
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</table>
Discussion

Cisplatin is a strong cellular toxin especially in the low chloride state. In the cell, the chloride atoms in cisplatin will be replaced by water molecules. This hydrolysis product is believed to be an active species that reacts with glutathione in cytoplasm and the DNA nucleus of renal tubular cell. More than 50% of these drugs are excreted in the urine within the first 24 hours after administration, platinum concentrations reach the renal cortex faster than plasma or other organs. Cisplatin primarily damages S3 segment of the proximal tubules causing a decrease in GFR 11.

Factors that increase the risk of nephrotoxicity include female, old age, smoking, hypoalbuminemia, and renal insufficiency. Age affects renal function because along with age it will also be followed by decreased renal function 12. Patients (>40 years) have a greater risk of decreased renal function. This is caused by nephron loss at that age. Decreased renal function estimation on the basis of each decade increase is about 10 ml/min/1.73 m². At the age of the fourth decade or 40 years there has been a decline in renal function about 10% of renal ability. Patients in advanced age are more susceptible to get drug side effects due to their physiological condition that has decreased 13.

Cisplatin nephrotoxicity can occur in a variety of signs and symptoms, one of the most commonly found is acute renal injury with the most common incidence of renal insufficiency. The onset of renal insufficiency occurs a few days after cisplatin consumption, which is indicated by increased creatinine serum and BUN levels. Cisplatin can cause damage to the renal blood vessels resulting in ischemia that may interfere with renal blood perfusion and consequently an increase in creatinine serum 14. Creatinine serum will increase significantly after 5 days of cisplatin administration. There will be a change in the partial glomerular atrophy of some tubular cells. The distal and proximal tubules exhibit partial degeneration and destruction of epithelial cells. Increased creatinine serum may be the final indicator of renal injury due to cisplatin chemotherapy because renal failure can occur without increase creatinine serum 15.

Hypomagnesemia is one of clinical manifestations that occur in patients who received cisplatin chemotherapy. Persistent exhalation of magnesium as a result of decreased magnesium levels indicates that hypomagnesemia occurs due to renal impairment in reabsorbing magnesium 4. Cisplatin causes magnesium receptors damage in the ascending branch of Henle curve and distal tubules, causing tubular cell necrosis resulting in disruption of magnesium reabsorption mechanism. It was found 1 (2.86%) patient who experienced post-chemotherapy hypermagnesemia. Hypermagnesemia is a rare condition except in renal failure or after parenteral Mg++ administration. This condition can cause central nervous system depression as well as heart function and sometimes hypermagnesemia itself may be an indication for dialysis 16.

Hypokalemia is a common electrolyte disorder occurring during cisplatin treatment, causing by increased renal reabsorption ability in response to reduced potassium absorption. Hypomagnesemia can also cause damage of Mg++ dependent Na, K-ATPase due to high loss of sodium in cells, if it was combined with a decrease in renal potassium causing hypokalemia 17. The results showed a significant decrease in glomerular filtration rate (GFR), significant increase in blood urea nitrogen (BUN) levels, significant increases in creatinine serum levels, significant decreases in magnesium (Mg++), and significant decrease in potassium (K+) levels. Thus the hypothesis of this study was proven 7.

Conclusion

There was a nephrotoxicity effect of 27.80% glomerulus filtration rate (GFR), increased blood urea nitrogen (BUN) level of 63.94%, increased serum creatinine of 41.80%, decrease magnesium (Mg++) level of 12.30%, decreased potassium (K+) level of 13% after cisplatin-paclitaxel chemotherapy treatment in patients with malignant head and neck tumors.

Ethical Clearance: This research process involves participants in the survey using a questionnaire that was accordant with the ethical research principle based on the regulation of research ethic committee. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, nonmaleficence, and justice.

Conflict of Interest: The author reports no conflict of interest of this work.

Source of Funding: This study is paid by authors’money only.
References


Correlation of CD4 and Distal Sensory Polyneuropathy in HIV-Aids Patients

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Abstract

Infection of human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) has developed into one of the most important health problems in the world. Distal sensory polyneuropathy (DSP) is the most common complication of peripheral neuropathy in HIV-AIDS. Risk factors associated with DSP are elderly, stage of HIV disease, (cluster differential-4) CD4 count, and high plasma viral load in plasma. To analyze the relationship between CD4 and distal sensory polyneuropathy in HIV-AIDS patients. Subjects of the study examined the degree of severity of distal sensory polyneuropathy by examination of the Surface Nerve Surgical Nerve Speed, then divided into case group and control group. After that, the subjects performed a CD4 count examination. Data analysis using chi-square test. Most body mass index (BMI) subjects did not experience malnutrition by 35% of case group and 35% control group (p = 1.000). Most subjects aged ≥40 years were 37.5% of case group and 25% control group (p = 0.102). Analysis of CD4 relation with Speed of Nerve Surgical Nerve Survival was obtained p = 0.038. There was a significant relationship between CD4 and Distal Sensory Polyneuropathy in HIV-AIDS patients. While age and malnutrition were not found a significant relationship with distal sensory polyneuropathy.

Keywords: CD4, distal sensory polyneuropathy, HIV-AIDS

Introduction

Currently, infection human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) has developed into one of the most important health problems in the world. In 2002, the number of new cases of HIV infection worldwide was reported at nearly 3.5 million and among 2.4 million cases of which died from AIDS 1. In 2003, 4.8 million newly infected individuals were infected with HIV. Centers for disease control and prevention (CDC) estimates that 40.000 individuals are infected with HIV each year. World Health Organization (WHO) estimates that the total number of people living with HIV/AIDS worldwide is 42 million 2. Data from the Ministry of Health of the Republic of Indonesia in the second quarter of 2010 increased by 1.206 cases of AIDS, thus as of June 2010 cumulative cases of AIDS reported since 1978 amounted to 21.770 from 32 provinces and 300 districts/cities. East Java was ranked second in HIV positive cases was 5.973 cases 3.

Along with the development of science and the existence of the drug is much active antiretroviral therapy better, then the life expectancy of patients is increasing, resulting in abnormalities in the peripheral nervous system that appears will increase. Peripheral neuropathy generally occurs early in HIV disease, but it can also occur in all stages of the disease with varying and complex clinical features 4.

Peripheral neuropathy (DSP), toxic neuropathies (TN), mononeuropathy multiplex (MM, including brachial and lumbar plexus), inflammatory demyelinating polyneuropathy (IDP, acute or chronic type), and progressive polyradiculopathy (PP). DSP is the most common complication and ranges one-third (about 35%) of all HIV patients 5.
DSP usually appears in later stages and is estimated to be 30% of patients with CD4 counts less than 200/μl. DSP is characterized by the degeneration of axons from sensory fibers with little regeneration of nerve fibers. Risk factors that correlate with DSP include elderly, HIV disease stage, CD4 lymphocyte cell count, and high plasma viral load in plasma, genetic factors may also play a role.6,7

Several factors are suspected to be associate with DSP in HIV patients. These factors include patient age, CD4 cell count, plasma HIV viral load, antiretroviral drugs, and the presence of diabetes mellitus. DSP in the study was upheld from clinical symptoms of the patient i.e sensory complaints in the distal. Neural conduction examination (NCS) with the electromyography device (EMG) was not performed in both studies although the sensitivity was 94% and the specificity was 62.1% for the case of polyneuropathy. Diagnosis of DSP is more objective and more precisely enforced through nerve conduction examination. However, there are some obstacles encountered: EMG checks are quite expensive, need trained operators, limited tools especially in peripheral areas, and possible risk of HIV transmission to other patients through micro lesions of EMG tools.8 Therefore, we aimed to analyze the relationship of CD4 with DSP in HIV/AIDS patients.

Method

The subjects of this study were HIV-AIDS patients who were treated at Dr. Soetomo Teaching Hospital Surabaya, Indonesia that fulfill the criteria of inclusion and exclusion. The subjects consisted of two groups: case and control group. Inclusion criteria are: patients diagnosed with HIV/AIDS, aged 17-60 years, had symptoms or asymptomatic Distal Sensory Polyneuropathy, and nerve survival rate (NSR) Survival (≤40 m/s in case group and ≥40 m/s in the control group)9,10. Exclusion criteria are: taking antiretroviral drugs (ARVs), HIV-AIDS patients using neurotoxic drugs such as Isoniazid, Metronidazol and chemotherapy drugs, and the subject had a history of Diabetes Mellitus. Subjects who were willing to participate in the research first fill out the informed consent sheet.

The procedure of determining the number of subjects includes the identification of HIV-AIDS positive patients using 3 methods of antigen examination for the HIV virus.9 HIV-AIDS patients performed NSR assessment using Electromyography or EMG (Cadwell Laboratories Inc., Kennewick, USA). The patients were then identified and divided into two groups: case and control group. The results of the process were obtained by 20 case group subjects and 20 control group subjects.

The design of this study using the method of case-control was performed by observational. This research procedure consists of several subject data retrieval including demographic and clinical data. Demographic data include sex, education, and age obtained by means of assessment on the subject. Clinical data include malnutrition and CD4 levels. Malnutrition was known by measuring BMI.11 Examination of CD lymphocyte T lymphocyte was performed by taking 2 ml of venous blood stored in EDTA vials. Further examined by flow cytometry method with BD FACS CaliburTM System and with BT Triset CD3 FITC/CD4 PE/CD45 PerCP reagent.

Before the identification of the subject, the researcher conducted a test of ethics at Dr. Soetomo Teaching Hospital Surabaya Indonesia. Data analysis in this research use frequency analysis which is categorized into 2 categories for each variable. Statistical data aliases using SPSS version 23.0 (SPSS, Inc., Chicago, IL). The statistical test used in this research was the chi-square test (p <0.05). If statistical test requirements are not met then the chi-square test is replaced by a Fisher test.

Results

The results of the assessment indicated that most of the subjects were male (52.5%) of which 32.5% of case group and 20% control group (p = 0.113). Most subjects aged ≥40 years (62.5%) were 37.5% of case group and 25% control group (p = 0.102). The majority of subjects had high school or college education (85%) of 42.5% case groups and 42.5% control group (p = 1.000; Table 1).
BMI results in subjects were found to be largely malnourished (70%) by 35% of cases and 35% of controls (OR = 1.000; CI 95%, p = 1.000). In this study, there was a statistically significant relationship between CD4 and surgeon nerve neural velocity with p = 0.038 and Odd’s Ratio of 4.636 (CI 95%, 1.023 - 21.004), meaning that subjects with low CD4 cell counts were <185 cells/mm³ has a risk of decreasing the Surface Nerve Surge Nerve Speed by 4.6 times compared to subjects with CD4 ≥185 cells/mm³. This indicates that the results of this study were clinically and statistically significant (Table 1).

**Discussion**

The prevalence of distal sensory polyneuropathy (DSP) is more prevalent in male patients than in female. The factor that causes the prevalence of DSP in the male is higher than for female was the prevalence of male HIV-AIDS patients in Indonesia was almost twice by female patients. In addition, higher male postures lead to more susceptible to length-dependent neuropathy such as DSP.

Older people have a higher risk of developing DSP than younger age. Study in the United States gained an average age of DSP over 45 years, whereas study in Kenya the median age of patients with DSP was 42 years. Moreover, the level of education is not related to NSR Nervus Suralis.

The condition of malnutrition may aggravate the condition of neuropathy in HIV/AIDS patients. In this study, malnutrition status was not related to DSP because malnutrition status is only assessed from Body Mass Index (BMI) ≤18.5. There was no significant association of DSP incidence with weight, height, and BMI. Malnutrition parameter not only obtained from body mass index but also assessed albumin level, hemoglobin level, and level of vitamin B12 in blood.

The CD4 cell count range in this study was 3 to 509 after a statistical analysis found a CD4 border that had a significant association with NSR Sural Nerve was 185. The CD4 count range for the occurrence of Distal Sensory Polyneuropathy between 50-199 cells/mm³. CD4 cell count <170 cells/mm³ had a significant association with the incidence of peripheral neuropathy.
in HIV/AIDS patients. HIV patients with a CD4 cell count <200 cells/mm³ had a risk of HIV neuropathy and HIV dementia was 3.5 times higher than patients with CD4 count >500 cells/mm³. Low CD4 cell counts indicate low immune system HIV/AIDS patients so that macrophage activation is increasing. High macrophage activation leads to the release of proinflammatory cytokines (TNF α, IL1, IL6). Excessive inflammatory cytokines accelerate the apoptotic process of neurons, especially sensory nerve fibers and cause DSP.

**Conclusion**

The most subjects were male, aged ≥40 years and the majority of subjects have a high school or Bachelor’s degree. The most subject was not malnourished and patients had CD4 <185 cells/mm³. There was a significant relationship between CD4 and Distal Sensory Polyneuropathy in HIV-AIDS patients. While age and malnutrition were not found a significant relationship with Distal Sensory Polyneuropathy.

**Ethical Clearance:** This study protocol was approved by ethical clearance Dr.Soetomo Teaching Hospital Surabaya, Indonesia.

**Conflict of Interest:** There is no conflict of interest in this study.

**Source of Funding:** This study is done with individual funding.

**Acknowledgement:** We are grateful to Faculty of Medicine for providing facilities and support. We thank the staff members for the participation during this study. All authors have made substantive contribution to this study and/or manuscript, and all have reviewed the final paper prior to its submission.

**References**


Legal Efforts on Forensic Examination of the Death of Polling Station Working Committees (KPPS) Members

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1Doctoral Student, Universitas Islam Indonesia

Abstract

Indonesia has held its general election on April 17th, 2019. During the 2019 general election, there were 11,239 members of polling station KPPS members passing out and 527 people passing away. Due to this circumstance, forensic examination is required to examine the reason behind the cases of the dead and the sick members. Therefore, this present article analyzes two issues: 1) how legal effort is conducted regarding health services provided for polling station KPPS members; and 2) what action the government do to take the responsibility on the death of the members.

This research is categorized as legal research that employs four approaches. The first approach is statuary approach by using ordinatie ssth No. 350 of 1957 Visum Et Repertum, preamble of 1945 State Constitution of Republic Indonesia article 179 of Criminal Code, Law of Health No. 36 of 2009 and Election Law No. 7 of 2017. The second one is case approach. The next is conceptual approach for both death and ill health cases. The last one is using comparative approach.

The result showed that the members of polling station committee died due to some illness suffered by the members such as cardiovascular, heart attack, stroke, or even all of them. However, for members who do not suffer from hereditary disease, the cause the death is due to the heavy workload they had that surpasses 20 until 22 hours a day. Besides, lack of sleep is also considered contribute to the members’ death and sickness.

Keywords: Forensic medicine, Polling Station Working Committee, 2019 General Election

Introduction

General election or Pemilu had been conducted in Indonesia on April 17th, 2019. It was one of the biggest democracy parties yet ever as this election includes presidential election and Indonesian legislative elections. Therefore, General Election Commission (KPU) and Regional Election Commission (KPUD) have plenty of things to prepare. Ballot papers, ballot boxes and polling booths are some election supports should be prepared. Besides, the commission also recruit many people to be appointed as polling station KPPS members (KPPS hereafter).

Due to the hectic 24-hour nonstop work and the heavy workload the KPPS members had prior to and after the election cause some people passing out, even many of them are confirmed pass away. According to research conducted by Sidiq Budi Sejati and Dewi Sendhikasari, there is a list of data issued by the KPU that showing the numbers of sick members and dead members. The data is listed as follow: (1)
<table>
<thead>
<tr>
<th>NO</th>
<th>PROVINCE</th>
<th>NUMBER OF SICK MEMBER</th>
<th>NUMBER OF DEAD MEMBER</th>
<th>TOTAL</th>
<th>NO</th>
<th>PROVINSI</th>
<th>NUMBER OF SICK MEMBER</th>
<th>NUMBER OF DEAD MEMBER</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ACEH</td>
<td>46</td>
<td>2</td>
<td>48</td>
<td>19</td>
<td>LAMPUNG</td>
<td>40</td>
<td>7</td>
<td>47</td>
</tr>
<tr>
<td>2</td>
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<td>5</td>
<td>20</td>
<td>MALUKU</td>
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<td>5</td>
</tr>
<tr>
<td>3</td>
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<td>17</td>
<td>6</td>
<td>23</td>
<td>21</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
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</tr>
<tr>
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<td>YOGYAKARTA</td>
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<td>5</td>
<td>7</td>
<td>13</td>
<td>EAST NUSA TENGGARA</td>
<td>8</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
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<td>24</td>
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<td>8</td>
<td>1</td>
<td>9</td>
<td>26</td>
<td>RIAU</td>
<td>25</td>
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<td>30</td>
</tr>
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<td>7</td>
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<td>2</td>
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<td>7</td>
<td>10</td>
</tr>
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<td>0</td>
<td>2</td>
<td>34</td>
<td>NORTH SUMATERA</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>17</td>
<td>RIAU ISLAND</td>
<td>4</td>
<td>1</td>
<td>5</td>
<td>5</td>
<td>TOTAL NUMBER</td>
<td>883</td>
<td>144</td>
<td>1027</td>
</tr>
</tbody>
</table>

Besides, Ministry of Health through regional public health offices records that KPPS members who got sick surpass 11,239 people and the dead members surpass 527 people. According to press release held by Ministry of Health in Jakarta on Wednesday (May 16th, 2019), as cited by Antara, those total numbers are generated from the investigation of Ministry of Health in 28 provinces per May 15th, 2019. Compared to this data, the dead members of polling station KPPS are increase, particularly in following four provinces: nine people in North Sumatera, four people in South Sulawesi, one person in Bangka Belitung, and one person in West Sulawesi.
Referring to the report made by Public Health Offices in each province, the highest number of dead KPPS members is in West Java province. For more complete data is provided b: 177 people in West Java(2). On the other hand, Ombudsman also released data of election related officers passing away during election period. However, the data is quite different. Ombudsman stated that there are 608 people including 486 KPPS members, 97 members of Election supervisory agency and 25 police officers who died. That is why Ombudsman conducts an assessment on the case by interviewing some officers of KPU, Regional KPU, Election Supervisory Agency, Ministry of Health, Indonesian Medical Association, and also the family members of the dead polling station working committee(3).

Concerning the aforementioned case, authors are interested to deeply analyze about Forensic Legal Efforts in health services towards the death case of polling station KPPS members during 2019 General Election. Specifically, there are two main issues that will be elaborated. The first is regarding the legal efforts in health services provided for KPPS members and the second one is related to governments’ responsibility in handling the death case of KPPS members.

Research Methodology

This study is a legal research in which four approaches are used, namely statutory approach (4), comparative approach, case approach and conceptual approach. Particularly, ordinantie sth No. 350 of 1957 Visum Et Repertum (report of an autopsy) is employed for the statutory approach. Meanwhile, comparing forensic medical theories with the death case of KPPS members during 2019 general election is employed as comparative approach to analyze the phenomenon.

Finding and Discussions

The field of medical forensic basically covers many components, namely forensic law, dentistry law, nursing law, clinical pharmacy law, hospital law, community health law, and environmental health law(5). Particularly for medical forensic, it defines some terms including post-mortem, mortem, ante mortem, and visum et repertum(6).

Prof. H.J.J. Leene defined medical forensic as medical science that provide direct health services. It relates to criminal laws and it uses sciences and literature as its legal sources. On the other hands, Prof. Van Derminjn stated that medical forensic is closely related to the management and the application of health in criminal law and administration law(7). Speculation sparks within society is that the polling station committee members are die unnaturally as they are poisoned by the political factions. Further discussion regarding the cause of the death of KPPS members will be elaborated in another article.

Forensic law is utilized when something unusual occurs regarding one’s condition that causes severe injury or death(8). In this case, forensic is required in order to know the cause of the death of polling station working members. Therefore, the authors are interested in analyzing deeply the concern regarding forensic employment.

In medical field, there are doctors who not only check the patients’ condition, but also assist police officers in examining one’s cause of death, particularly the unusual one(6). Such doctors who have to contend with dead patients are called as forensics doctors. Specific skills and competencies in uncovering patient’s cause of death is definitely required(9). It is regulated in Article 179 of Indonesian Criminal Code as stated as follows: “(1) everyone who is asked for his/her opinion as medical/ juridical expert or as doctor or other expert is obliged to provide expert information for the sake of justice”. Therefore, doctors’ obligations are categorized into several classifications.

1. Based on the object of examination: live people, corpse, organ or parts of body.
2. Based on the service: conduct examination and provide opinion on the result of examination, only provide opinion without conducting examination.
3. Based on the working place: in hospital/laboratory, in the crime scene, and in the court.

Examination concerning cause of death is necessary in order to prove whether someone is suicide, homicide/murdered, poisoned or etc. In this case, Visum Et Repertum shall be taken place. Basically, there are two types of Visum Et Repertum. They are:

a. Visum Et Repertum on body or corpse: it is conducted to know an odd cause of death due to criminal issue through autopsy
b. Visum Et Repertum on injured patients:
1) Patients who do not need particular treatment after examination: outpatients, autopsy on types of wound, trauma, and injury classification.

2) Patients who decline medical treatments after examination: types of trauma, description on treatment decline even though need some.

Autopsy report can be utilized as a proof in police investigation report to prove the criminal occurrences. It not only covers the examination result of the victims, but it also should be reported in written form. Following is how to write Visum Et Repertum:

a. Write it on the stamped paper with word “Pro Justitia” written in left corner and use general and logical language.

b. Do not use abbreviation on any sentences, numbers or symbols.

c. Any incorrect parts should be signed by the authorities’ officers by providing correction statement.

d. Type the result of Visum Et Repertum neatly and it should be sealed(10).

Pro Justitia

Visum Et Repertum

1. Introduction: it states the identity of all involved parties; people who are being examined; officers who examine the case; time and place where the examination takes place; and modus operandi.

2. Examination part: it states whether the object being examined is dead or alive and provides statement that can be seen or perceived as the expert information that is used as a real proof.

3. Conclusion part: it states the opinion of the examining doctor; causes of death, damage, or negligence. In this part, a judge is allowed not to take side with the suggested opinion.

4. Closing part: According to ordiantie sth No. 350 of 1957, Visum Et Repertum is closed or finalized by such oath or promise when accepting the position”.

The result of Visum Et Repertum is submitted to the authorities. However, according to the applicable law, it does not state the exact time of the submission. Visum Et Repertum is usually required during investigation in which police officers need the post mortem result as one of undeniable proof(11).

In the case of the polling station KPPS members, the autopsy result is used by the family or police to find out whether or not intentional acts that lead to criminal acts are involved. Autopsy examination is expected to provide brief result regarding the cause of the death. It may also give a clue for the investigator teams to examine the case. If the result of Visum Et Repertum indicates any crime issues, the death case can be proceeded to the court (12).

Even though there are hundreds of victims, none of the family members give permission for the authorities to conduct Visum Et Repertum. They said that it possibly has been their destiny. The same thing goes to the family members of the injured committee. They refuse to conduct the autopsy and only give oral statement regarding the case. Conducting general election requires the involvement of polling station KPPS members (KPPS). Unless the committee involvement, the election will not run as expected. However, not everyone could easily appointed as polling station working committee. The requirements are strictly regulated on Law No. 7 of 2017 on General Elections. To form polling station working committee, seven adults living near the polling station. The selection of committee members is conducted fairly by considering their competencies, capacities, and integrity(13).

Researchers from Universitas Gajah Mada investigated the cause of death of polling station KPPS member during 2019 general election. The researchers observed 400 polling stations out of 11,781 available polling stations throughout the Special Region of Yogyakarta. The result showed that from 400 dead members of polling station KPPS member, 12 of them are coming from Special Region of Yogyakarta. This result is reported in a press release in KPUs, Jakarta on June 25th, 2019. The more detail results obtained by the researchers are elaborated as follows:

1. The primary cause of the death of polling station KPPS members is not due to poisoning, but due to some illnesses formerly suffered by the committee members. The illnesses include cardiovascular disease such as heart attack, stroke, or both of them. Other illnesses is excessive fatigue (20%), severe dizziness (12%), low back pain (10%), shortness of breath (5%), etc. As for the sick members, 29 percent of the available members suffered from 1 up to 3 of deadly diseases.
2. Technical problems, logistics, and members’ health problems formerly suffered trigger the members’ illness and the death case. Moreover, lack of sleep leads to excessive fatigue. Prior to the election period, the workload is four times higher (80%) than the break time (20%). This percentage actually decreases as the Election Day approaching. One day prior to the Election Day, the workload is more than twice higher (70%) than the break time (30%) while one day after the election, the workload is little bit higher (60%) than the break time (40%).

3. The researchers found that the polling station KPPS members had experienced multiple morbidities or repeatedly suffering from the same disease. Heavy workloads around 20 until 22 hours a day also worsen the situation. Besides, 80% of the dead members formerly suffered from cardiovascular disease that relates to heart and blood vessels’ condition. Further investigation also revealed there are some other factors that also contribute to the members’ death. They are psychological factor and tiredness (70%), anxiety (30%) and physical condition (40%).

4. The researchers suggest KPPUs to invite students taking community service program to be recruited as polling station KPPS members to lift the heavy workload of the KPPS member. However, certain mechanism to be considered in order to control the quality of the work[14].

Government’s responsibility on constitutional law is included in the principle of good governance. Regarding this issue, government should take responsibility on the case by covering all medical expenses for the ill committee members and giving death benefit for the family of dead members.

Moreover, government is required to investigate the case thoroughly. Considering the high number of dead members of polling station working committee, further solution for the next election should be reckoned by providing comprehensive health services and workers for the KPPS. Furthermore, government through Ministry of Health could perform thorough examination to investigate the cause of the death. Following that, the result may be announced to the public through mass media. Besides, further information regarding death benefit and medical expenses coverage should be clearly stated[15].

Conclusion

Legal efforts through forensics examination on polling station KPPS member had been attempted by conducting visum et repertum. However, such attempt is declined by the KPPS family. The examination result is only deduced from family’s verbal testimony. The researchers from Universitas Gajah Mada revealed that the cause of the death of polling station KPPS is due to cardiovascular disease such as heart attack, stroke, or both of them that formerly suffered by the KPPS members. According to verbal autopsy result, committee members with no hereditary diseases get sick or die due to excessive fatigue and heavy workload that surpasses 20 until 22 hours a day on several day.

Ethical Clearance : Yes
Conflict of Interest: No

Source of Funding: Authors

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The Burden of Toxoplasma Gondii In Spontaneous Miscarriage and its Association with Rhopty Protein 5 Gene and Toxoplasma gondii specific primers GRA 6 Gene Expression in Iraq Women

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¹Wasit University/College of Medicine/ Iraq

Abstract

Background: One of the major infectious micro-organism that is associated with spontaneous abortion is toxoplasmosis. Under diagnosis of toxoplasmosis and lack of understanding the exact mechanism and pathogenesis of spontaneous abortion associating toxoplasmosis are in our opinion the main problem facing clinicians who are concerned with categorization and management of spontaneous abortion in our pregnant ladies.

Aim of the current study: to diagnose placental tissues parasitic infections using sophisticated nested PCR method in addition to evaluating gene expression of two pathogenetically Toxoplasma genes, namely ROP-5 and GRA-6, as a step toward estimating the exact prevalence of Toxoplasma associated spontaneous abortion in Iraqi community

Patients and methods: The case control study was based on the inclusion of 101 women with spontaneous abortion and 20 pregnant ladies who succeeded to get pregnant serving as control group. The placental tissues were obtained from the pool of women visiting Al-Kut maternity teaching hospital for purpose of delivery or because of abortion. Those tissues were then processed in central laboratory using conventional PCR technique searching for evidence of toxoplasma gondii DNA. Obtained tissues were also subjected to real time PCR for detection of Rhopty protein 5 gene expression and Toxoplasma gondii specific primers GRA 6. The lab work was done according to instruction of providing company.

Results: Positive results were limited to women with spontaneous abortion, that is, none of control women had PCR evidence of toxoplasma DNA in their placental tissues, 72 (71.3 %) versus 0 (0.0 %); the difference was highly significant (P < 0.001). An estimation of the risk of abortion accompanying toxoplasmosis has been carried out in terms of approximate Odds ratio and the results has been strikingly high (100.8). Both Rhopty protein 5 and Toxoplasma gondii specific primers GRA 6 gene expressions have been significantly correlated to serum IgG and IgM positive tests in a negative way.

Conclusion: PCR carried out on placental tissue is significantly higher sensitive in detecting toxoplasma gondii than serology and Both Rhopty protein 5 and Toxoplasma gondii specific primers GRA 6 gene expressions play significant role in spontaneous abortion.

Key words: spontaneous abortion, Rhopty protein 5, Toxoplasma gondii specific primers GRA 6 gene expressions

Introduction

The problem of spontaneous abortion is relatively common in our community as well as in several regions around the world (¹). The problem of spontaneous abortion...
abortion in addition to infertility account for the major obstacles that every newly married couple may face during their planning to build up their families (2). In addition, spontaneous abortion is associated with a number of obstetric complications that may increase the incidence rate of morbidity and mortality accompanying natural pregnancy (3). Examples of these complications are retained placental and or fetal parts with risk of bleeding and infection. Indeed, hemorrhage and sepsis are among common associated complications that may threaten the life of every pregnant lady experiencing spontaneous abortion (3). Therefore, identifying causes and risk factors that may precipitate spontaneous abortion is a crucial step in the prevention and treatment of spontaneous abortion and its associated complications (4).

Infection of the product of abortion is by far one of the principal etiological factors that predispose pregnant ladies to spontaneous abortion. One of the major infectious micro-organism that is associated with spontaneous abortion is toxoplasmosis (5). Indeed, toxoplasmosis is both common worldwide and in our community (5). This parasite is responsible for a number of morbidities and mortalities as it can infect a number of human systems and tissue such as nervous system and pregnancy products (6).

Serological investigations are the routine investigations that are carried out in daily obstetric practice in order to diagnose toxoplasmosis in women with recurrent spontaneous abortions (7). However, the sensitivity and specificity of these serological methods are variable and are far less than that offered by sophisticated molecular investigations (8).

Under diagnosis of toxoplasmosis and lack of understanding the exact mechanism and pathogenesis of spontaneous abortion associating toxoplasmosis are in our opinion the main problem facing clinicians who are concerned with categorization and management of spontaneous abortion in our pregnant ladies (9). For that reasons, the following study was designed, planned and carried out in order to diagnose placental tissues parasitic infections using sophisticated nested PCR method in addition to evaluating gene expression of two pathogenetically Toxoplasma genes, namely ROP-5 and GRA-6, as a step toward estimating the exact prevalence of Toxoplasma associated spontaneous abortion in Iraqi community and to clarify the role of these two genes in the pathogenesis of spontaneous abortion accompanying toxoplasmosis.

**Patients and Method**

The case control study was based on the inclusion of 101 women with spontaneous abortion, mostly in the first trimester and some in the second trimester, and 20 pregnant ladies who succeeded to get pregnant serving as control group. The placental tissues were obtained from the pool of women visiting Al-Kut maternity teaching hospital for purpose of delivery or because of abortion. Those tissues were then processed in central laboratory using conventional PCR technique searching for evidence of *toxoplasma gondii* DNA. Obtained tissues were also subjected to real time PCR for detection of Rhopty protein 5 gene expression and *Toxoplasma gondii* specific primers GRA 6. The lab work was done according to instruction of providing company.

The statistical work was carried out using SPSS version 23 and Microsoft Office Excel 2010. Categorical variables were expressed as number and percentage whereas quantitative variables were expressed as mean and standard deviation. Independent sample t-test was used to compare mean age between study and control groups and chi-square was used to study association between categorical variables. Risk was estimated according to odds ratio and 95 % confidence interval. The level of significance was considered at *P* ≤ 0.05.

**Results**

The statistical analysis in the current study was based on the inclusion of 101 women with spontaneous abortion, mostly in the first trimester and some in the second trimester, and 20 pregnant ladies who succeeded to get pregnant serving as control group. The mean age of women with spontaneous abortion was 29.10 ±7.35 years and that of control group was 25.70 ±5.75 years; despite some difference in mean age, there was no statistical significance (*P* = 0.053). The results of nested PCR results, that have been performed on women retrieved placental tissues, are shown in table 1. Positive results were limited to women with spontaneous abortion, that is, none of control women had PCR evidence of *Toxoplasma* DNA in their placental tissues, 72 (71.3 %) versus 0 (0.0 %); the difference was highly significant (*P* < 0.001), as shown in table 1. The lab work was done according to instruction of providing company.

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strikingly high (100.8). This implies that any women with placental tissue, toxoplasma invasion is 100 times more liable to develop abortion than women whom placental tissue is free of toxoplasmosis, table 1.

**Table 1: Comparison of nested PCR test results between study and control groups**

<table>
<thead>
<tr>
<th>Nested PCR</th>
<th>Abortion group n = 101</th>
<th>Control group n = 20</th>
<th>$\chi^2$</th>
<th>P €</th>
<th>Approximate odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>72 (71.3 %)</td>
<td>0 (0.0 %)</td>
<td>35.207</td>
<td>&lt; 0.001 HS</td>
<td>100.8</td>
</tr>
<tr>
<td>Negative</td>
<td>29 (28.7 %)</td>
<td>20 (100.0 %)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n: number of cases; €: Fischer exact test; HS: Highly significant at $P \leq 0.05$

Rhoptry protein 5 gene expression in placental tissues obtained from study group is demonstrated in 1. Gene expression up regulation has been observed in 66 (91.5 %) of cases. Gene expression of *Toxoplasma gondii* specific primers GRA 6 in placental tissue of study group has been shown in table 3.13 and figure 3.4. Gene expression down regulation has been observed in 25 (34.7 %); whereas, gene expression up regulation has been recorded in 24 (33.3 %) of cases, as shown in table 3.13. Significant negative association has been observed between the expression of ROP5 and GRA6 as shown in figure 3.4.

**Figure 1**: Rhoptry protein 5 gene expression and *Toxoplasma gondii* specific primers GRA 6 in placental tissues obtained from study group

Both Rhoptry protein 5 and *Toxoplasma gondii* specific primers GRA 6 gene expressions have been significantly correlated to serum IgG and IgM positive tests in a negative way (negative correlation coefficients, -0.347 and -0.374, respectively and a $P$ value of $< 0.01$), as shown in table 2, implying that the higher the level of serum IgM and IgG, the lower the opportunity for *Toxoplasma gondii* to proliferate and invade placental tissue.

**Table 2: Correlations of Rhoptry protein 5 gene expression and *Toxoplasma gondii* specific primers GRA 6 to clinical parameters**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ROP5</th>
<th></th>
<th>GRA6</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
<td></td>
<td>P</td>
</tr>
<tr>
<td>Age</td>
<td>0.042</td>
<td>0.727</td>
<td>-0.033</td>
<td>0.781</td>
</tr>
<tr>
<td>Residency</td>
<td>0.084</td>
<td>0.484</td>
<td>-0.085</td>
<td>0.476</td>
</tr>
<tr>
<td>Parity</td>
<td>0.024</td>
<td>0.838</td>
<td>-0.122</td>
<td>0.309</td>
</tr>
<tr>
<td>Previous abortions</td>
<td>0.028</td>
<td>0.818</td>
<td>0.127</td>
<td>0.288</td>
</tr>
<tr>
<td>Gravidity</td>
<td>0.040</td>
<td>0.740</td>
<td>0.045</td>
<td>0.710</td>
</tr>
<tr>
<td>Trimester</td>
<td>0.142</td>
<td>0.236</td>
<td>0.052</td>
<td>0.666</td>
</tr>
<tr>
<td>IgG</td>
<td>-0.347</td>
<td>0.003</td>
<td>-0.087</td>
<td>0.465</td>
</tr>
<tr>
<td>IgM</td>
<td>-0.374</td>
<td>0.001</td>
<td>-0.215</td>
<td>0.070</td>
</tr>
</tbody>
</table>
Discussion

Although, PCR can be performed on blood as well as serum samples, positive results are usually low, moreover, the presence of positive PCR results using blood or serum is an indirect evidence for a causal relationship between toxoplasmosis and spontaneous abortion (10); whereas, positive nested PCR results from placental tissues of aborted mothers will be a direct evidence for such causal relationship. For these reasons, the authors of the current study preferred to perform nested PCR on placental tissues over PCR on blood and serum samples.

In the current study, the use of nested PCR significantly increased the detection rate of Toxoplasma parasite in women with spontaneous abortion from 41.6 % to 71.3 % in comparison with serological investigation. In addition, the sensitivity and specificity of both IgG and IgM were low in comparison with nested PCR method. In one study, carried out in Iran on 200 women with spontaneous abortion, the sensitivity of serological test was 53.5 % while that of nested PCR using placental tissue was 10.5 % (8), in disagreement with finding of the current study. Indeed, the low sensitivity and specificity of serologic technique using anti-toxoplasma IgG and IgG in comparison with nested PCR using placental tissue has been proved by several other authors (11), (12) and (13) in clear agreement with our findings.

Toxoplasma gondii is an obligate intracellular single-celled parasite that can invade all warm-blooded animals worldwide. During invasion, proteins from parasite organelles such as rhoptry proteins (ROPs) and dense granule proteins (GRAs) are released into host cells and are able to cause significant host damage (14) and (15). Importantly, maternal Toxoplasma infection may give rise to congenital transmission of the parasite to the fetus through the placenta and/or via interfering with the immune tolerance on maternal-fetal interface (16), (17), (18) and (19).

Toxoplasma gondii, as many intracellular parasites, is separated from the cytosol of its host cell by a parasitophorous vacuole membrane (PVM). This vacuole forms during host cell invasion and parasite apical organelles named rhoptries discharge proteins that associate with its membrane during this process. ROP5 is not processed during trafficking to rhoptries. We show here that ROP5 protein with vacuole membrane can be indirectly assessed by studying ROP5 gene expression in placental tissues of women with spontaneous abortion. Molecular investigation in the current study showed upregulation of ROP5 gene expression up regulation has (91.5 %) of cases.

Both Rhopty protein 5 and Toxoplasma gondii specific primers GRA 6 gene expressions have been significantly correlated to serum IgG and IgM positive tests in a negative way (negative correlation coefficients, -0.347 and -0.374, respectively and a P value of < 0.01), implying that the higher the level of serum IgM and IgG, the lower the opportunity for Toxoplasma gondii to proliferate and invade placental tissue.

Ethical approve: Our study was approved by institutional ethical approval company. Following task facilitating request from my organization (College of Medicine, Wasit University) verbal consent was officially obtained from Wasit Health institution all participating women.

Source of Funding: Self-funded

Conflict of Interest: Nil.

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Changes of Interleukin-6 (IL-6) and Immunoglobulin G (IgG) in Respiratory Exercise

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Abstract

Background: Skeletal muscle contraction in exercise will synthesize and release Interleukin-6 (IL-6) into the systemic circulation. IL-6 is a pleiotropic, proinflammatory and anti-inflammatory cytokine. IL-6 in exercise can modulate the immune response and metabolism in the liver, adipose tissue, hypothalamus-hypophyse-adrenal (HPA). Objectives: To analyze and prove changes in levels of Interleukin-6 (IL-6) and Immunoglobulin G (IgG) in respiratory art sports of a exercise club. Methods: Thirty-four members of the breathing arts sport who met the inclusion criteria, male sex, adult age (≥21 years), willing to participate were enrolled in this study. Examination of IL-6 and IgG levels were performed by using Elisa method. The kit for IL-6 used Human IL-6 Immunoassay and IgG level used Total Human IgG Assay. Results: The t-test results obtained p = 0.027 (p <0.05), there was a change between IL-6 pre-test and IL-6 post-test of breathing exercise for three weeks. The t-test results obtained p = 0.000 (p <0.05) there was a change between IgG level of pre-test and IgG post-test of breathing arts sports treatment, this change increased significantly. Conclusion: There was a change in levels of IL-6 and IgG in the breathing exercise.

Keywords: Adipose tissue, breathing exercise, IgG, IL-6

Introduction

Physical, emotional, and environmental challenges are some of the stressors of human life and this results in a physical response of mild to severe illness. The influence of globalization and the current economic pressures has changed the lifestyle and socio-economic community. This change makes people become stressed and lack of movement and lack of exercise. This leads to an increased risk of metabolic and degenerative diseases such as heart disease, kidney, lung, hypertension, diabetes, obesity, and immune disorders.

The impact of stress on physical health can be reduced by exercising regularly. Sports health is a physical activity to maintain health and improve body condition. Respiratory exercise in mild hypoxia conditions can stimulate the increase of various cytokines and stimulate more strongly as stress immunologic response compared to other sports. Breathing exercises of Satria Nusantara with special methods are: the breath, movement, and concentration (pray, spiritual, meditation) which is a physical exercise, mental and social exercise. Satria Nusantara breathing exercise can improve physical fitness and improve body immunity.

Skeletal muscle is the largest organ in the human body. Skeletal muscle contractions in exercise will synthesize and release myokines or cytokines called Interleukin-6 (IL-6) to the interstitial and systemic circulation. This myocyte or cytokine affects the metabolism, the nervous system, the endocrine system, and the immune system that plays a role in the maintenance of homeostasis. Contraction of skeletal muscles when a person is exercising can contribute greatly to the levels of the IL-6 present in the circulation.

Increased levels of IL-6 during exercise include levels of other cytokines, such as IL-1 receptor antagonists (IL-1ra), Tumor necrosis factor receptor (TNF-R), and IL-10. The magnitude of elevated levels of IL-6 induced by exercise response depends on the intensity, duration and model of exercise.
synthesis of IL-6 in muscle contraction occurs through mechanisms: increased calcium influx, hepatic glucose availability, and increased formation of Reactive oxygen species (ROS), this will activate transcription factors governing IL-6 synthesis.

Interleukin-6 (IL-6) is a pleiotropic, proinflammatory and anti-inflammatory cytokine. IL-6 during exercise can modulate immune and metabolic responses in the liver, adipose tissue, Hypothalamus-pituitary-adrenal (HPA) and leukocytes. IL-6 plays a role in the differentiation of B lymphocytes into plasma cells that produce immunoglobulins.

Immunoglobulins are glycoproteins that obtained in blood and other body fluids, which contain antibodies and are produced to protect the body against pathogens. Antibodies formed in the primary immune response are generally Immunoglobulin M (IgM), whereas in the secondary immune response is Immunoglobulin G (IgG). IgG is the highest level of immunoglobulin, which is up to 50-80% of all immunoglobulins in the body. Immunoglobulin levels in serum are used to assess the integrity of immune system function.

Satria Nusantara’s Respiratory Breathing exercise mechanism to increase body immunity until now has not been thoroughly studied, especially the change of IL-6 and IgG levels in Satria Nusantara’s breathing arts sport is still unclear and require further study. The study aimed to determine the changes levels of IL-6 and IgG in Respiratory Exercise.

**Method**

This study was a laboratory study of quasi-experimental analysis and type with one group pretest-post-test design without the control group (comparison) by measuring levels of Interleukin-6 (IL-6) and Immunoglobulin G (IgG) between before and after treatment breathing sport Satria Nusantara.

The study was conducted in the sports arts community of Satria Nusantara Surabaya. Laboratory examination of IL-6 and IgG levels were performed by Elisa method in Department of Clinical Pathology, Faculty of Medicine, Dr. Soetomo Teaching Hospital Surabaya. The study was conducted for 5 months, beginning with library search until presentation of research report. Treatment is conducted in August to September 2013.

The sample used was the member of the sport by 34 members of the sport of breathing art, the basic level of hard control, previously had regular practice 2x/week regularly, with the intention did not require the stage of introduction of motion stance first because it was accustomed and have mastered the breath, stance, concentration correctly, and meet the criteria of the sample.

Inclusion criteria are respiratory sports artist Satria Nusantara Surabaya branch, adult age (≥21 years), male gender, regular practice 2x/week regularly, and willing to participate in the research. While for the exclusion criteria are members who have coronary heart disease, unregulated diabetes mellitus, suffering from systemic infectious diseases, suffering from immunodeficiency, suffering from malignancy, receiving immunosuppressive and antioxidant therapy. The subjects declared drop-out if not exercising more than 1 time out of 9 exercises during the treatment program to be followed or if the absence of more than 12%.

Working procedure begins with members who have met the inclusion criteria carried out the recording of basic data in the form of the name, age, gender, address. Members who are willing to be subjects of research then sign a consent letter following research and medical action as well as medical action information. Prior to the treatment of Satria Nusantara’s breathing art program, blood sampling was performed through the vein mediana kubiti. Blood sampling prior to treatment to obtain baseline values of IL-6 and IgG levels. After that, the research subjects follow the treatment of sports art program Satria Nusantara breathing, for 3 weeks, optimal frequency 3 times a week with duration 90 minutes once the practice. If the last exercise was completed, immediate blood retrieval was performed no more than 1.5 hours after treatment, this is because the half-lives of IL-6±1.5 hours.

Examination of IL-6 levels was performed using Elisa method. The kit used is Human IL-6 Immunoassay, Quanticine Elisa from R & D Systems. The examination of IgG level was performed by using Elisa method. The kit used is Total Human IgG Assay, Elisa kit from Diagnostic Automation, Inc. USA.

All the collected data was arranged in the data collection sheet in tabular form and processed statistically using SPSS 22.0 (SPSS. Inc. Chicago IL). Univariate
data analysis was used to describe each variable, either independent or dependent variable from case group and control group with frequency distribution table. Furthermore, two-tailed paired t-test two paired t-test.

**Results**

Characteristics of the sample consist of age, weight, height and body mass index (BMI). The number of samples obtained in this study were 34 samples, with a sample characteristic of 34 men (100%). The mean age 50.680 years with SD (±10.2590) and age range between 37-73 years old. Weight has a mean of 66.353 kilograms with standard deviation (SD) (±11.0189) and the weight range between 43.0-93.0 kilogram. The mean height (mean) in this study was 164.574 centimeters with SD = (±7.5218) and a height range between 147.5-176.0 centimeters. Body mass index (BMI) has mean of 24.3874 kg/m² with SD = (±2.8568) and BMI range between 19.53-30.37 kg/m² (Table 1).

<table>
<thead>
<tr>
<th>Variables (N=34)</th>
<th>Mean ± Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Characteristics of Research Subject</strong></td>
<td></td>
</tr>
<tr>
<td>Age(Year)</td>
<td>50.680 ± 10.2590</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>66.353 ± 11.0189</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>164.574 ± 7.5218</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>24.387 ± 7.5218</td>
</tr>
<tr>
<td><strong>Physical Examination Data</strong></td>
<td></td>
</tr>
<tr>
<td>Systole blood pressure (mmHg)</td>
<td>117.940 ± 6.2910</td>
</tr>
<tr>
<td>Diastole (mmHg)</td>
<td>76.470 ± 4.8510</td>
</tr>
<tr>
<td>Pulse frequency(x/minute)</td>
<td>78.590 ± 1.6720</td>
</tr>
<tr>
<td>Frequency of breath (x/minute)</td>
<td>19.060 ± 1.7220</td>
</tr>
<tr>
<td>Body temperature (0C)</td>
<td>36.868 ± 0.2239</td>
</tr>
<tr>
<td>HR max(x/minute)</td>
<td>169.320 ± 10.2590</td>
</tr>
<tr>
<td>75% HR max (x/minute)</td>
<td>127.150 ± 7.6920</td>
</tr>
<tr>
<td><strong>IL-6 levels</strong></td>
<td></td>
</tr>
<tr>
<td>IL-6 pretest</td>
<td>5.098 ± 1.9528</td>
</tr>
<tr>
<td>IL-6 posttest</td>
<td>6.044 ± 2.0311</td>
</tr>
<tr>
<td><strong>IgG levels</strong></td>
<td></td>
</tr>
<tr>
<td>IgG pretest</td>
<td>447.351 ± 228.5582</td>
</tr>
<tr>
<td>IgG posttest</td>
<td>821.559 ± 177.7086</td>
</tr>
</tbody>
</table>

Note: IL-6: Interleukin-6, IgG: Immunoglobulin G
Physical examination is performed before the subjects follow the training program. This physical examination was performed by measuring blood pressure, breath frequency, temperature, and pulse frequency. The results obtained as follows mean systole blood pressure 117.94 mmHg with SD (± 6.2910) and systole blood pressure range between 110-130 mmHg. While diastole blood pressure 76.47 mmHg with SD (±4.8510) and diastole blood pressure range between 70-80 mmHg. The mean pulse rate was 78.59 x/min with SD (±1.6720) and a pulse frequency range between 76-80 x/min. The mean breath frequency (sample) in this study was 19.06 x/min with SD = (±1.7220) and breath frequency range between 76-80 x/min. The mean body temperature of the sample in this study was 36.868 °C with SD (±0.2239) and the temperature range was between 36.5-37.0 °C (Table 1).

The intensity of the specified workload load is submaximal, determined by the Target heart rate (THR), in a manner calculated with 70% max HR. While maximal HR was calculated with the maximal HR formula = (220-age). The maximum mean frequency of samples was 169.32 x/min with SD (±10.2590) and maximum HR range between 147-183 x/min. The average frequency of THR or 75% HR was 127.15 x/min with SD (±7.692) and maximal 75% HR range between 110-137 x/min (Table 1).

The mean IL-6 pre-test level was 5.0918 pg/mL with SD (±1.95284) and the mean IL-6 level was 6.0444 pg/mL with SD (±2.0311) (Table 1). The mean rate of pre-test IgG 447.3509 mg/dL with SD (±228.5582) and mean post-test IgG was 821.5597 mg/dL with SD (±177.7086) (Table 1).

Table. 2 Two-sided test results (2-tailed)

<table>
<thead>
<tr>
<th>Variable (N=34)</th>
<th>T</th>
<th>df</th>
<th>Sig.(2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 levels</td>
<td>-2.309</td>
<td>33</td>
<td>0.027</td>
</tr>
<tr>
<td>IgG levels</td>
<td>-8.642</td>
<td>33</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Note: p<0.05

Levels of IL-6 have a difference of pre-test and post-test mean is (0.95265). The t-test H0: μ pre-test = μ post-test, gives the value t = (-2.309) with degrees of freedom = (n-1) = (34-1) = 33. The SPSS output gives the p-value for two-tailed test = (0.027) (Table 2). Two-tailed t-test results proved that there was a change between IL-6 pre- and IL-6 post-test of breathing exercises of Satria Nusantara art for 3 weeks, the change was significantly increased (p <0.05).

While the mean difference of IgG level of pre-test and post-test was (374.2089). From the table Paired sample test also obtained d = (- 374.2089) and Sd = (252.4772) (Table 2). Two-tailed t-test results proved that there was a change between pre-test IgG and post-test IgG post-breathing exercises of Satria Nusantara respiratory art for 3 weeks, this change was significantly increased (p <0.05).

**Discussion**

Based on two-tailed paired t-test analysis of parameters of IL-6 and IgG parameters between pre- and post- there is a change so that this result is in accordance with some previous research which states if respiratory sports satria nusantara can increase physical fitness and body immunity 5,9. However, other studies suggest that the study was conducted on male students of the 2nd grade Madrasah Aliyah Mu’alimin Yogyakarta who met the inclusion criteria, the result that respiratory exercise of Satria Nusantara can increase beta-endorphin, IgG and IL-6 levels, while IL-2 and IL-4 did not increase, and cortisol levels decreased 9.

Increased levels of IL-6 pre-test and IL-6 post-test treatment of breathing art Satria Nusantara. The resulting increase in IL-6 levels is due to increased IL-6 secretion during exercise physical exercise. IL-6 can increase to 100-fold in physical exercise 7. The IL-6 response was sensitive to exercise intensity, indirectly representing the large muscle mass involved in contraction. Exercise that only involves limited muscle mass, was not sufficient for improving IL-6. Exercise involving large muscles resulted in significant increased IL-6. Increased IL-6 during exercise can be inhibited by antioxidant vitamins C and E as well as drugs Indomethacin (class of NSAIDs). This regimen will inhibit NF-kB activity and inhibit elevated levels of IL-6 in response to exercise 10.

IL-6 might cause some effects in some tissues. IL-6 has catabolic properties, shown by the ability to increase energy expenditure, increase lipolysis, increase fat oxidation, increase endogenous glucose output (by reducing insulin signals in fat and liver), and increase cortisol. The apparent difference between tissues regarding the response to IL-6 may be due to IL-6 signals in different tissues. IL-6 which was released from muscle contraction can stimulate an anti-inflammatory response.
reflected increased IL-1ra, IL-10, CRP, and cortisol without elevated pro-inflammatory mediators 7,11.

Serum IgG levels in individuals who were given physical exercise for 45 minutes (acute moderate exercise) increased levels of IgG and IgM. This increase was due to extravasation blood proteins, increased lymph flow, noradrenergic sympathetic neural influences on immune responses, or many immunogens inhaled along with respiratory air, whose flow increases during exercise, and the destruction of the mucosal immune response that dries up during exercise. This level decreases after 1.5 hours of exercise and when the exercise is increased with a marathon or run of 45-75 km (ultramarathon) then the second level of the immunoglobulin decreases 12.

Other studies have suggested that runners athletes will decrease cadres of IgM, IgG and IgA after undergoing heavy exercise 13. However, it was different from other studies suggesting that short rest periods during exercise will not suppress IgA secretion, but increase cortisol levels. Exercise with moderate and regular intensity will provide a good effect for the immune system with the occurrence of increased immunoglobulin. It was because exercise with a heavy intensity will suppress some parameters of the immune system, among others: Ig A, IgG, and IgM 14. Several other studies have also suggested that weight training until exhausted in the morning and evening has a significant effect on the humoral immune system (IgG, IgA, and IgM) and serum cortisol 15.

Exercise sports a pleasant breathing art will nourish the body and also improve body immunity. The existence of abdominal pressing and abdominal pressing during the motion exercise motion of Satria Nusantara, as an effort to guarantee the implementation of breath arrest during the movement. Abdominal pressing does not cause a disturbance of physiology, and in contrast to thoraco-abdominal pressing (manoeuvre valsava) which can cause physiological disturbance and harm because it will raise blood pressure at the beginning of pressing, followed by decreased blood pressure due to retardation of reverse blood flow and venous pressure will increase. In preliminary exercises need to be explained and emphasized the difference between respiratory chest with abdominal breathing in exercise Satria Nusantara 16,17.

Abdominal breathing will smooth the blood flow back from the vein in the abdominal area to the heart, inspiration time (sucking breath) pressure in the abdomen increased and the pressure in the chest cavity decreased. Increased pressure inside the abdominal cavity causes a sort of massage to the organ in the abdominal cavity, a mechanical stimulation of the digestive tract of food, and will improve peristaltic movement. Abdominal breathing also trains the respiratory muscles and abdominal wall muscles 16,17.

**Conclusion**

There were changes in levels of Interleukin-6 (IL-6) and Immunoglobulin G (IgG) in the breathing art of Satria Nusantara. Increased levels are still within the normal range and good in improving body immunity.

**Ethical Clearance:** This study protocol was approved by ethical clearance Dr.Soetomo Teaching Hospital Surabaya, Indonesia.

**Conflict of Interest:** The author reports no conflict of interest of this work.

**Source of Funding:** This study is done with individual funding.

**References**

5. Sudarko RA. The changes in the level of beta endorphin, intersleukin-2, interleukin-4, interleukin-6, immunoglobulin and cortisol hormone on the practices of satria nusantara.


Association between Stretching Exercise with Virtual Reality Game and Over Head Pulley of Frozen Shoulder Patients

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Abstract

Background and Objective: Frozen Shoulder (FS) or adhesive capsulitis is the most common musculoskeletal condition that causes pain and extensive restriction of joint motion. Virtual Reality (VR) provides a new option that is expected to help the rehabilitation program. The objective of the study was to compare the effect of stretching exercises with virtual reality games (VGR) and Over Head Pulley (OHP) through shoulder joint motion range (JMR) improvement and functional ability of FS.

Method: This research was conducted on July to September 2012, in Medical Rehabilitation Outpatient Unit of Dr. Soetomo General Hospital Surabaya. The samples were frozen shoulder patients. These inclusion criteria were new or old unilateral FS patients, the limitations of shoulder JMR both passive and active, with a minimum limit of 30 ° compared to normal shoulder JMR at least 2 shoulder movements, flexion and abduction between 6 weeks to 6 months, aged 35-65 years.

Result: The One-Sample Kolmogorov-Smirnov Test on the age range obtained no significant differences (p = 0.418), the chi-square test on the sexes showed no significant differences (p = 1.000). The median of pleasure after treatment with the Mann-Whitney Test showed a non-significant difference (p = 0.317).

Conclusion: Stretching exercises compared with VGR exercises in FS patients provided the same benefits of stretching with the OHP on improvement.

Keywords: Frozen Shoulder, Overhead pulley, Virtual Reality, shoulder wheels

Background

Frozen Shoulder (FS) or adhesive capsulitis is the most common musculoskeletal condition that causes pain and extensive restriction of joint motion range (JMR) which interferes ¹. The exact pathophysiology of FS remains unknown and FS occurs mostly when the disuse factor occurs in people with stress, anxiety, and apathy accompanied by low pain thresholds. Effective standard therapy to restore JMR and eliminate the pain of FS has not been found yet. Previous studies have demonstrated 90% successful of conservative therapy with oral NSAID and rehabilitation therapy. Generally, FS therapeutic principles are medicaments (Non Steroid Anti Inflammatory Drug/NSAID, muscle relaxants, and steroid injections), modalities therapy (cold, heating, transcutaneous electrical nerve stimulation), exercise therapy (JMR exercises such as overhead pulley (OHP), shoulder wheel, finger ladder, Codman Pendular exercise), shoulder manipulation, and surgery ².

A variety of new methods and techniques in the rehabilitation of FS have been developed such as dynamic splinting rigid or Kinesio taping. Exercises with shoulder wheels, OHP, and sticks are standard exercises for FS. Other studies have shown significant improvements in the use of pulleys with transcutaneous
electrical nerve stimulation compared to thermal therapy modalities with therapeutic exercise and manipulation. The OHP is used as one of the standard rehabilitation therapy for stretching exercises in dealing with FS patients in sub-acute or chronic phase.

Virtual Reality (VR) is an interactive environment produced by computer simulations with the real world. This technology is run through an interface that has been adapted to human senses, thus making the user into the virtual world. Users can react with the virtual world through every action detected from position and movement, therefore the hardware processes changes that occur in the virtual world according to the rules created by its creators and provide feedback to its users. Initially, VR was used in students with physical disorder who were often excluded from research because of their lack ability resulting the difficulty to control themselves when in dangerous situations.

Thus, this new technology could provide an attitude-enhancing experience and reduce anxiety. In its development, VR was applied to various rehabilitation programs such as stroke, head trauma, autism, cerebral palsy, and rehabilitation of cognition. The VR has potential to achieve compliance required for effective rehabilitation. Side effects of VR were nausea and visual impairment, but this effect on many studies was minimum or nonexistent. The VR provides a new option to help the rehabilitation program, although it still requires further research and evaluation.

This study was a preliminary study to determine the effect of stretching exercises with virtual reality games (VRG) on the improvement of shoulder JMR and functional ability in FS patients, compared to stretching exercise with OHP which became standard therapy in Medical Rehabilitation, with attention to patient’s pleasure. Therefore, stretching exercises with VRG can be used as a new choice therapy in the treatment of FS patients in the future.

**Method**

This research was an experimental research, randomized pre-post test control group design done on FS patients. This research was conducted on July to September 2012, in Outpatient Unit of Medical Rehabilitation Installation of Dr. Soetomo General Hospital Surabaya. The samples were FS patients who met the inclusion criteria and did not meet exclusion criteria. Ethical appeals were submitted to the Ethics Commission for basic science/clinical research at Dr. Soetomo Surabaya.

The inclusion criteria were new or old unilateral FS patients, with JMR limitation both passive and active, having minimum limit of 30° compared to normal shoulder JMR at least 2 shoulder movement such as flexion and abduction between 6 weeks to 6 months, aged 35-65 years, able to read the clock, could understand and follow simple verbal instructions, willing to fill out shoulder self-report form in the instrument of DASH, mild shoulder pain with VAS score 1-3, the strength of the affected side shoulder muscles: MMT ≥4, willing to stop therapy under treatment with replaced therapy available in the study, willing to participate in this study by signing an informed consent after getting an explanation.

The samples were randomized into 2 groups, group 1 (control) received ultrasound diathermy modal therapy on painful shoulder for 10 minutes and shoulder stretching exercise using OHP, with frequency 3-9 times a week. Group 2 (treatment) received ultrasound diathermy at shoulder pain area for 10 minutes and shoulder stretching exercises using VRG, with frequency 3-9 times a week.

The data were tabulated and analysed statistically using SPSS (SPSS. Inc. Chicago IL). The hypothesis test for increased shoulder JMR obtained ratio data thus independent t-test, delta, paired t-test were performed for the groups. Hypothesis test of functional ability improvement (DASH) pre and post test obtained ordinal data thus it was done Wilcoxon signed rank test. The functional abilities between the treatment and control groups were performed by Wilcoxon-Mann Whitney test and the delta pre and post treatment. The pleasure level obtained ordinal data thus it was done Wilcoxon signed rank test.

**Result**

The average of shoulders JMR of flexion, extension, abduction, adduction, external rotation and internal rotation after treatment between control and treatment groups showed no significant difference (p >0.05). The result of abnormal JMR delta on abduction in control group between before and after treatment was 27.5° ± 33.5° (p = 0.053). While in treatment group was 15.0° ± 12.2° (p = 0.010) (Table 1).
The result of average JMR delta on abduction between the control group and the treatment group showed a non-significant difference ($p = 0.338$, Table 3). While on flexion in the control group was $11.3 \pm 13.6^\circ$ ($p = 0.051$). The average delta on flexion in the treatment group was $10.0^\circ \pm 11.0^\circ$ ($p = 0.037$). The average delta on flexion between control group and treatment group showed significant difference ($p = 0.843$, Table 1).

Median pain scores before and after treatment between the control group and the treatment group showed no significant differences ($p > 0.05$). Median pain scores before and after treatment in the control group showed no significant differences ($p = 0.066$). Median pain scores before and after treatment in the treatment group showed a significant difference ($p = 0.034$) (Table 2).

The median delta pain score in the control group was 0.5 and in the treatment group was 1.0 with $p = 0.955$ (Table 4). Median score of shoulder functional ability, both before and after treatment between control group and treatment group was $p > 0.05$ (Table 2). The median delta score of functional ability in the control group was 9.05 and in the treatment group was 12.50; with the Mann-Whitney Test showed no significant difference ($p = 0.792$) (Table 4). The median pleasure level after treatment between control group and the treatment showed a non-significant difference ($p = 0.317$, Table 3).

### Table 1. Comparison of Shoulder JMR Before and After Treatment

<table>
<thead>
<tr>
<th>JMR</th>
<th>Group</th>
<th>Pre</th>
<th>Post</th>
<th>Changes</th>
<th>Comparison test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>($x \pm SD$)</td>
<td>($x \pm SD$)</td>
<td>($x \pm SD$)</td>
<td>(Pre-post)</td>
</tr>
<tr>
<td>Abduction</td>
<td>Control</td>
<td>91.9o ± 21.7o</td>
<td>119.4o ± 39.5o</td>
<td>27.5 o ± 33.5 o</td>
<td>$p = 0.053$</td>
</tr>
<tr>
<td></td>
<td>Group</td>
<td>90.6 o ± 31.4o</td>
<td>105.6 o ± 37.5o</td>
<td>15.0 o ± 12.2 o</td>
<td>$p = 0.010$</td>
</tr>
<tr>
<td></td>
<td>Comparation test (between groups)</td>
<td>$p = 0.928$</td>
<td>$p = 0.487$</td>
<td>$p = 0.338$</td>
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<tr>
<td></td>
<td>Control</td>
<td>110.6 o ± 20.6o</td>
<td>121.9 o ± 19.1o</td>
<td>11.3 o ± 13.6 o</td>
<td>$p = 0.051$</td>
</tr>
<tr>
<td>Flection</td>
<td>Treatment</td>
<td>91.9 o ± 32.4o</td>
<td>101.9 o ± 36.9 o</td>
<td>10.0 o ± 11.0 o</td>
<td>$p = 0.037$</td>
</tr>
<tr>
<td></td>
<td>comparison test (between groups)</td>
<td>$p = 0.189$</td>
<td>$p = 0.195$</td>
<td>$p = 0.843$</td>
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</tr>
</tbody>
</table>
Table 2. Comparison between Pain and Functional Ability (DASH) before and after group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Pre (Median)</th>
<th>Post (Median)</th>
<th>Changes (delta)</th>
<th>Comparison (Pre-post) test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain (Score)</td>
<td>Control</td>
<td>3.0</td>
<td>2.0</td>
<td>-0.5</td>
<td>p = 0.066</td>
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<tr>
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<td>Treatment</td>
<td>2.0</td>
<td>1.5</td>
<td>-1.0</td>
<td>p = 0.034</td>
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<tr>
<td></td>
<td>Comparison (between groups)</td>
<td>p = 0.206</td>
<td>p = 0.427</td>
<td>p = 0.955</td>
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<tr>
<td>Shoulder Functional Ability (Score)</td>
<td>Control</td>
<td>20.45</td>
<td>13.60</td>
<td>-9.05</td>
<td>p = 0.018</td>
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<tr>
<td></td>
<td>Treatment</td>
<td>23.90</td>
<td>14.75</td>
<td>-12.50</td>
<td>p = 0.018</td>
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<td></td>
<td>Comparison (between groups)</td>
<td>p = 0.673</td>
<td>p = 0.711</td>
<td>p = 0.792</td>
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</tbody>
</table>

Table 3. Comparison of Pleasure Level after Treatment between Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Score (Median)</th>
<th>Comparison Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pleasure</td>
<td>Control</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>4.0</td>
<td>p = 0.317</td>
</tr>
</tbody>
</table>

Discussion

Frozen shoulder often occurs on non-dominant shoulder because when the shoulder hurts, the body trying to protect by bringing the shoulder to the body and tends not to use the sick shoulder. Other studies have shown that humans who stand upright in their daily activities use supraspinatus muscles, thus gravity causes stress in capsules and tendons that retain hanging arms, and forward and sideways movements cause friction and compression resulting in rotators cuff muscle ischemia between tuberosity major and acromion. OHP has an assistive active stretching effect, indicated in FS sufferers who have subtle signs, where active practice without help is ineffective. Patients with FS who have subtle signs are patients who can not reach the ears or even the back of the head by using a sore shoulder.

Almost all subjects in this study were included in the subtle sign. In control group, when subjects moved the healthy shoulder and the sick shoulder was indirectly pulled by the pulley, thus he sick shoulder muscles did not work fully and there was a maximum pull. In accordance with the theory that the pain is the biggest inhibitor for a muscle movement. This should be avoided, especially in the shoulder area, because its muscle activity required coordinated, stable and functional joint movement. It was proven in previous studies that there was a delay in muscle latency in the impingement subjects versus the healthy group. this was similar to a study on knee that reported an onset of vatus laterals happened before vastus medialis indicating the presence of different motor control.

In addition, because the strain treatment group had to respond to the order of the VRG first, thus the stretching
time was less. Reduced time of this strain would affect the effect. Based on guidance from the American College of Sports Medicine (ACSM), stretching prescribing prescriptions had to adhere to the following guidelines, those were 3 times a week, the intensity at mild discomfort, 10-30 seconds for each stretch, and 3 to 5 repetitions for each stretch

The improvement of JMR in the treatment group due to active stretching exercises was consistent with a study that reported the success of rigid shoulder therapy with active JMR practice. Other studies have shown that active JMR pendulum and exercise were better than passive stretches and manipulations that exceed the pain threshold.

Increased shoulder JMR and functional abilities in this study, proving that physical therapy (modalities and stretching exercises) significantly reduced pain and increased shoulder JMR in patients with FS. Thermal modality therapy as one of FS procedures before exercise was more effective to regain JMR and restore function, but stretching exercise was a major component of therapy in musculoskeletal disorders. A prospective study on the effect of shoulder stretching programs of 75 patients with stage 2 FS showed that 90% subjects had satisfactory results and only 7% required further therapy.

Previous research on healthy subjects was given a pain stimulus while playing VRG which was evaluated directly by functional Magnetic Resonance Imaging reported that there was a significant decrease in pain rate and an increase in brain oxygen activity. Possible mechanism of analgesics was through the attention diversion from pain stimuli and an increase in the pleasure level.

This pleasure would produce endorphins through limbic system activity. Endorphins played a role as excitation-conducting agents that activated brain analgesia system. Pain in treatment group obtained a significant decrease after treatment. Other studies found that adherence to rehabilitation therapy was associated with improved therapeutic outcomes. The VR had the potential to help achieve the adherence required to undergo an effective rehabilitation program. Given the increased motivation through the enjoyment and enthusiasm of the patient’s compliance with physical rehabilitation therapy, VR provided additional useful physical therapy for patient in rehabilitation program.

Both groups did not get any side effects either due to modalities or stretching exercises. Exercise should be done gently and with concentration. The subjects had to understand the purpose of the exercise, thus the effort was more focused and avoided the substitution movement. Uncomfortable sensations might occur, but not to interfere, or to cause persistent pain and aggravation. Stretching exercises were performed with a ‘stretched’ sensation, not a pain.

**Conclusion**

It could be concluded if the stretching exercise compared to JMR exercise in FS patients, VRG provided the same benefits of stretching with the OHP on improvement. Stretching exercises with VRG provided similar benefits compared to stretching exercises with OHP on shoulder functional abilities in FS patients. Stretching exercise therapy with VRG and OHP gave the same pleasure value in FS patients. Stretching exercise therapy with VRG could reduce pain.

**Conflict of Interest** : The authors report that there is no conflict of interest related with this paper.

**Source of Funding** : This study is done with authors’ funding only.

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Correlation of Serum Alkaline Phosphatase, Lactate Dehydrogenase, C-Reactive Protein, Blood Deposition Rate, B-Hcg Expression and Tumor Volume to Lung Metastasis Risk in Osteosarcoma Patients

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Abstract

Osteosarcoma is the most common type of sarcoma found in bone. The survival rate in osteosarcoma patients was less than 20.00% at the end of 1980 but is currently increasing to over 70.00% for nonmetastatic patients. The objective to analyze the correlation between Lactate Dehydrogenase (LDH), Alkaline Phosphatase (ALP), C-Reactive Protein (CRP), Blood Deposition Rate (LED), hCG expression and tumor volume with pulmonary metastases in osteosarcoma patients. The subjects were osteosarcoma patients that the data taken from January 2015 to December 2016. The subjects will be clinical, radiological (plain, CT scan thorax, MRI) examinations, serum marker tests consisting of ALP, LDH, CRP and LED and histopathologic staining IHC β-hCG. The study design was cross-sectional, while the statistical analysis used was normality test, Spearman correlation, chi-square test and logistic regression. The results majority of subjects were male (52.00%) and aged 11-20 years (85.00%). The tumor volume (p = 0.07), LDH levels (p = 0.07), and ALP levels (p = 0.016) were statistically significant for pulmonary metastases with moderate correlation (r = 0.587; 0.587; 0.53). The tumor volume was the most sensitive biomarker to diagnose lung metastasis in osteosarcoma patients (p = 0.171). Conclusion among other protein biomarkers, tumor volume was most sensitive in diagnosing pulmonary metastases. The volume of the tumor will increase the risk of pulmonary metastases in osteosarcoma patients.

Keywords: Serum Alkaline Phosphatase, Lactate Dehydrogenase, C-Reactive Protein, Blood Deposition Rate, B-Hcg Expression And, Tumor Volume To Lung Metastasis.

Introduction

Osteosarcoma is a malignant neoplasm and is the most common type of sarcoma found in bone ¹. Before the discovery of chemotherapy regimens in the late 1980s, survival rates in patients were less than 20.00%, and up to now have increased to over 70.00% for nonmetastatic patients. Currently, osteosarcoma diagnostics have been developed using biomarkers such as Alkaline phosphatase (ALP), Lactate dehydrogenase (LDH), C-reactive protein (CRP), Erythrocyte sedimentation rate (ESR), Beta human chorionic gonadotropin (β-hCG), and tumor volume².

ALP is a member of zinc metalloproteinase that is produced and located in the membrane cell of osteoblasts. Osteoblast cell transformation in osteosarcoma impairs strict control of proliferation and progressively causes constant increases in ALP levels ³. The association between total ALP activity and the clinical condition of osteosarcoma patients has been known for more than 50 years. However, studies related to the role of ALP levels against prognostic osteosarcoma are still inconsistent⁴.

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LDH is an enzyme involved in anaerobic metabolism of neoplasms. Many retrospective studies of serum LDH as prognostic factor survival rates in patients with osteosarcoma. However, the results are not convincing. Some studies show poorer results in patients with high LDH levels, while others are not.

CRP is a systemic biomarker widely used to diagnose acute and chronic inflammation. The measurement of serum CRP is a simple, inexpensive, and available method in day-to-day practice. It can serve as an additional prognostic predictor for survival and post-care monitoring in cancer patients.

Erythrocyte sedimentation rate (ESR) is one of the most common laboratory tests used in determining a progression in malignancy. Earlier research mentioned that in the case of patients with ESR increases by 25.00% suffered malignancy, but did not have significant statistics with the incidence of malignancy.

Human chorionic gonadotropin (hCG), a heterodimer protein comprising non-covalent bonds between the α and β subunits, usually secreted by the placenta. Several reports indicate that serum β-hCG and tissue levels are independent prognostic factors for adverse outcomes in some types of tumors. However, negative correlations were also found, with no association between β-hCG levels and survival of patients. Therefore researchers were interested in analyzing serum levels of LDH, ALP, CRP, LED and β-hCG as biomarkers to pulmonary metastases in osteosarcoma patients.

Method

The subjects were osteosarcoma patients that data was taken from January 2015 to December 2016. The inclusion criteria were patients with a clinical and radiological diagnosis of an osteosarcoma and an age less than 25 years. Exclusion criteria were patients who refuse care and research. The study design was cross-sectional. Subjects will be taken history data (age and sex) and physical examination. Subjects also performed laboratory tests in the form of LED, CRP, ALP, LDH. The LED was measured using the Westergren method with a normal value of 15-20 mm. Normal ALP values for males were 45-115 U/l and females 37-98 U/l. The normal standard CRP was ≤10 mg/l with the Immunoturbidimetric Assay method. The normal value of LDH was 100-190 units/l using the Beckman Unicel® DxC 800 Synchron method. The subjects also performed the radiological examination with MSCT thorax (metastatic thorax) and MRI (Volumetric tumor).

The subjects will also be examined histopathologically starting with immuno histochemical imaging on the biopsy specimen. The tissue block was cut with a thickness of 6 micrometers and placed on glass slides, followed by depolarization with xylene and rehydration gradually using an alcohol solution and washed in Tris-buffered saline solution with Tween 20. The specimen block was then immersed in the target retrieve solution, and placed in the hot water bath for 20 minutes and cooled for 20 minutes. Then incubated with β-hCG antibody for 30 min at room temperature on moisture chamber.

Data analysis using test of normality test then continued with correlation test with spearman. Statistical analysis of β-hCG using chi-square test. The quantitative data groups include tumor volume, ALP, LDH, CRP and LED correlated with data groups with nominal data scale, i.e., pulmonary metastases using logistic regression and followed by multivariate analysis to determine which variables are most sensitive as lung metastasis biomarkers. Statistical analysis program using SPSS (SPSS, Inc., Chicago, IL).

Results

Characteristics of subjects

The majority of subjects were male (52.00%) and aged over 11-20 years (85.00%). In the histopathology sample, 17 patients were obtained from open biopsy preparation and post-amputation tissue. 1 patient died before the procedure was performed and 2 patients lost to follow-up and decided not to continue treatment. Out of the 17 specimens obtained, 2 preparations were excluded because the lysis cells were painted (Table 1).

Additionally, 73.00% subjects showed positive results of expression of β-hCG with the majority of subjects of 64.00% with osteosarcoma chondroblasts type. The majority of subjects who expressed negative β-hCG of 50.00% with chondroblastist osteosarcoma type. Out of the 11 positive subjects expressing β-hCG, there were 64.00% of subjects who did not have metastasis to the lungs otherwise negative subjects β-hCG, 50.00% of subjects each metastasized to the...
lungs and did not have metastases to the lungs (Table 1).

**Bivariate Research Analysis**

The tumor volume (p = 0.07), LDH level (p = 0.07), ALP level (p = 0.016) was statistically significant for pulmonary metastases with moderate correlation (r = 0.587; 0.587; 0.53). While CRP levels, plasma levels, and β-hCG expression did not have significant statistics with pulmonary metastases (p = 0.471; 0.447; 1.000) (Table 2).

**Table 1. Characteristics of Research Subject**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Amount (N=20)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>7</td>
<td>35.00</td>
</tr>
<tr>
<td>Male</td>
<td>13</td>
<td>65.00</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-10 and/or</td>
<td>1</td>
<td>5.00</td>
</tr>
<tr>
<td>11-20 and/or</td>
<td>17</td>
<td>85.00</td>
</tr>
<tr>
<td>21-30 and/or</td>
<td>2</td>
<td>10.00</td>
</tr>
<tr>
<td>β-hCG Expression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>11</td>
<td>55.00</td>
</tr>
<tr>
<td>No</td>
<td>4</td>
<td>20.00</td>
</tr>
<tr>
<td>Unevaluated</td>
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<td>25.00</td>
</tr>
<tr>
<td>Lung Metastases</td>
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</tr>
<tr>
<td>Yes</td>
<td>7</td>
<td>35.00</td>
</tr>
<tr>
<td>No</td>
<td>13</td>
<td>65.00</td>
</tr>
<tr>
<td>Location of Tumor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distal Femur</td>
<td>13</td>
<td>65.00</td>
</tr>
<tr>
<td>Proximal Tibia</td>
<td>4</td>
<td>20.00</td>
</tr>
<tr>
<td>Distal Tibia</td>
<td>1</td>
<td>5.00</td>
</tr>
<tr>
<td>Proximal Fibula</td>
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<td>5.00</td>
</tr>
<tr>
<td>Proximal Humerus</td>
<td>1</td>
<td>5.00</td>
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<tr>
<td>Type Osteosarcoma</td>
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<td></td>
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<tr>
<td>Chondroblastic</td>
<td>11</td>
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<tr>
<td>Fibroblastic</td>
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<td>10.00</td>
</tr>
<tr>
<td>Osteoblastic</td>
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<td>5.00</td>
</tr>
<tr>
<td>Fibroblastic dan Osteoblastic</td>
<td>2</td>
<td>10.00</td>
</tr>
<tr>
<td>Chondroblastic dan Osteoblastic</td>
<td>1</td>
<td>5.00</td>
</tr>
<tr>
<td>Drop out/Die</td>
<td>3</td>
<td>15.00</td>
</tr>
</tbody>
</table>

**Independent Logistic Regression Analysis of Independent Variables Research**

The result of the analysis showed that p-value sub-variable of tumor volume (0.055), LDH (0.17) and ALP (0.136) had the p-value <0.25, so it entered the multivariate test. The smallest value of significance was obtained in tumor volume (p = 0.171). So the tumor volume was a predictor of the sensitivity factor to determine the presence of pulmonary metastasis (Table 2).

$\beta$-hCG = Beta-Human chorionic gonadotropin
**Table 2. Correlation between Independent Variable and Lung Metastasis (N=20)**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Spearman test (N=20)</th>
<th>Chi-Square Test</th>
<th>Logistic Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p-value</td>
<td>r</td>
<td>p-value</td>
</tr>
<tr>
<td>Volume Tumor</td>
<td>0.07</td>
<td>0.587</td>
<td>-</td>
</tr>
<tr>
<td>LDH level</td>
<td>0.07</td>
<td>0.587</td>
<td>-</td>
</tr>
<tr>
<td>ALP level</td>
<td>0.016</td>
<td>0.53</td>
<td>-</td>
</tr>
<tr>
<td>CRP level</td>
<td>0.471</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LED level</td>
<td>0.447</td>
<td>-0.180</td>
<td>-</td>
</tr>
<tr>
<td>Expression β- Hcg</td>
<td>-</td>
<td>-</td>
<td>1000</td>
</tr>
</tbody>
</table>

**Discussion**

Assessment of tumor malignancy degree based on ALP gives a moderate correlation. The meta-analysis study showed that patients with high ALP values were significantly correlated with a high risk of having osteosarcoma metastasis at the time of diagnosis (Ren, Sun et al. 2015). Serum ALP in patients showed a higher value of metastatic events in patients with higher ALP levels in diagnosis initials than patients with low ALP levels.

Assessment of malignancy degree based on LDH examination in this study showed statistically significant with moderate correlation. This was consistent with a study showing that the percentage of patients with metastases, having twice the LDH (36.60%) higher than non-metastable (18.80%) patients 9.

Assessment of malignancy degree based on LED rate gives negative correlation value. In this study of 20 patients only obtained a significant increase in LEDs in 1 patient (with >4x normal values). In a previous study, the value of an increase in LEDs was only 25.00% of all patients with malignancy 6. In general, the 2 main factors that play a role in increasing the LED was the aggregation of erythrocytes and hematocrit. Aggregation of erythrocytes was influenced by plasma protein levels resulting in decreased negative electrostatic pressure among red blood cells that causing aggregation and increased sedimentation. If variability was found in the composition and interaction of plasma proteins then different values will be obtained.

Assessment of malignancy degree based on CRP examination gives negative correlation. Inflammation was the first sign of initiation and progression of cancer, where there was a linkage between intrinsic factors (oncogenes, genome instability) and extrinsic factors (immune factors and tissue around the tumor) 10. CRP was one single molecule to monitor acute phase reactions. CRP was a sensitive marker but not specific to inflammation. CRP was an independent prognostic factor for survival in high-grade osteosarcoma. The role of CRP as an acute phase protein and the prolonged time span between symptom emergence and diagnosis can be a factor in the absence of a positive correlation between CRP levels and the incidence of pulmonary metastases.

The significance of β-hCG IHC staining in tumor cells was unclear. In this study obtained a positive result 55.00% of the total samples performed β-hCG painting. A recent study showing 57.00% of a total of 49 patients had positive expression and no correlation between β-hCG expression and clinical outcome in patients 11. The results obtained can also be attributed to the limited
time span of the study, and the samples obtained within the timeframe are few. Further research was needed to find out the correlation of β-hCG expression with the outcome and pulmonary metastases with longer periods of time and a large number of samples.

The tumor volume was the best predictor of pulmonary metastases. If after being given tumor chemotherapy increased or fixed, it can be said to have a therapeutic response is still bad. The tumor volume had a sensitivity value of 68.6% and an 80.2% specificity for detecting pulmonary metastases. Patients with a >15 cm diameter tumor had a 3.4 times higher risk of death, whereas in patients with tumor diameter <15 cm the survival rate was better. In patients with small tumor, volumes showed a better response to therapy, with a positive predictive value of 88.00%.

**Conclusion**

However, serum CRP, LED, β-hCG expression did not have a statistically significant relationship with pulmonary metastases. What affects these three biomarkers did not correlate that can limited time span of research, samples, and plasma protein levels. The tumor volume was the best predictor factor against the risk of pulmonary metastasis in osteosarcoma patients. Further studies include larger and longer-term samples so that β-hCG expression may affect pulmonary metastases in osteosarcoma patients.

**Conflict of Interest:** There is no conflict of interest

**Source of Funding:** This study is self-funded

**Ethical Clearance:** This study was approved by Ethical Comission of Health Research Faculty of Medicine University of Airlangga

**References**


Effect of Amniotic Membrane-Derived Mesenchymal Stem Cells on TNF-α Expression and Inflammatory Cells Infiltration during Vesicovaginal Fistule Repair Healing Process

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Abstract

Background: Vesicovaginal fistula (VVF) causes high morbidity in women, affecting both physical and psychological condition. Until now, surgery is the mainstay treatment for this condition. However, prolonged exposure during inflammatory phase after surgery is still a problem in the healing process. TNF-α as potent pro-inflammatory cytokine plays an important role by attracting inflammatory cells to wound tissue. Amniotic membrane is the source for mesenchymal stem cells that had anti-inflammatory and immunomodulatory effect. This study aims to evaluate the effect of Amniotic Membrane-Derived Mesenchymal Stem Cells (AMMSC) on TNF-α expression and inflammatory cell infiltration during VVF repair healing process in New Zealand White (NZW) rabbit model.

Method: This study was an experimental study with randomized posttest only control group design. Twenty-seven NZW rabbit as VVF model was used in this study, randomly divided into 3 different treatment groups after underwent surgical treatment (no treatment <C group>, treated with freeze-dried amniotic membrane <T1 group>, and treated with freeze-dried amniotic membrane that seeded with AMMSC <T2 group>). Evaluation was done 7 days after treatment. TNF-α expression was evaluated semiquantitatively using modified Remmele-Stegner scale. Inflammatory cell infiltration was evaluated using modified Klopfleisch method.

Results: Mean TNF-α expression between C, T1, and T2 group were significantly different (8.5 ± 1.6; 7.1 ± 1.2; 1.6 ± 1.2 respectively, p < 0.001). Median inflammatory cell infiltration between C, T1, and T2 group were significantly different (3.0; 2.0; 1.0 respectively, p < 0.001).

Conclusion: AMMSC significantly reduced TNF-α expression and inflammatory cells infiltration during VVF repair healing process.

Keywords: vesicovaginal fistule, amniotic membrane mesenchymal stem cells, TNF-α, macrophage, neutrophil

Introduction

Vesicovaginal fistula (VVF) causes high morbidity in women, affecting both physical and psychological condition. Quick and accurate diagnosis followed by timely repair is essential to the successful management of these cases. Surgery with primary sutting are still the treatment of choice despite the challenge in healing process and to reduce the recurrence rate. The key to achieve satisfactory surgical outcome for VVF is by
having a good visualization of the surgical field, adequate necrotic tissue dissection, tissue approximation, and the drainage of urine. Surgical access could be transvaginal or transabdominal, depending on the location, size, and profile of the area around the fistula. Another important factor that affect the success of the surgery is the surgeon’s experience.

Failure in VFF healing after fistula surgery is complex and influenced by the severity of the surrounding tissue. Recent systematic review found that the success rate in VFF healing after surgery in developed countries are 94.6%, and 80.4% in patients with cervical malignancy after radiation. Recurrence was reported three months after the surgery with an average failure on day 25. In developing countries, VFF recurrence occurred around 41%. Healing wounds in urogynaecology system require a longer process than in skin because of the influence of spongiosum tissue around the wound and pro-inflammatory cytokine that alter the cell migration. The potent pro-inflammatory cytokine which plays a major role is tumor necrosis factor alpha (TNF-α). TNF-α will stimulate the inflammatory process, attract neutrophils and macrophages to clean up debris around the wound. Once this phase is passed for 4 days, then progressed to proliferative phase. When the inflammatory phase occurs for longer period, it will disrupt and impede the wound healing process.

Urogynaecology tissue engineering had promising result as a potential therapy by reconstructing tissue engineering and tissue regeneration using a combination of biomaterials, stem cells and other biomolecules. Stem cells have the property of self-renewal, differentiation and immunomodulation. It can be given as monotherapy or combined with tissue framework (scaffold) serves to support the cell to attach and differentiate. Local injection of stem cella in experimental animals showed good results. This gives hope to the future in the management of urogynaecology cases.

Human amnion is one source of biomaterials and stem cells. As a biomaterial, amnion can be used directly to close the defect wounds or as scaffold. The one used is dried amnion as a source of stem cells and resemble mesenchymal stem cell (MSCs). The amnion has low immunogenicity properties, antimicrobial and anti-inflammatory effect. It is able to express growth factor as well as proteins such as collagen, glycoproteins, integrins, and lamellar body that helps cells to grow and proliferate. The additional effect is immunomodulation of amniotic stem cells and anti-inflammatory. This study aims to evaluate the effect of dry amniotic and seeding amniotic membrane mesenchymal stem cell (AMMSc) on the expression of TNF-α and inflammatory cell infiltration in VVF using animal model.

**Method**

This study was an experimental analytic study with randomized posttest only control group design. This study was conducted at the Tropical Disease Center Airlangga, Surabaya on March-May 2017. Samples in this study was female New Zealand White (NZW) rabbits with inclusion criteria as follows: weigh 3-4.5 kg, aged 3-5 months, and without birth defect or scar. Dropout criteria for this study were rabbits whose died during the study period.

There were 27 rabbits used in this study. All rabbits were designed to had VVF by making a 5 mm defect on the walls of the vagina and the urinary bladder via laparotomy. Vaginal and urinary bladder wall was then sewn with thread SAFIL 4-0 HR 22 (BI Braun, Tutlingen, Germany), then the defect is maintained by Naso Gastric Tube (NGT) 16F for 3 weeks. Prior to surgical procedure, subjects were under anesthesia using ketamine 25-40 mg/kg and azepromazine 0.25-1 mg / kg intramuscular. After the procedure, tolfenamic acid 10 mg / kg / day were given for post-surgical pain reliever.

After 3 weeks, subjects were divided into 3 groups using simple random sampling, namely control (C) group, treatment 1 (T1) group, and treatment 2 (T2) group. C group underwent laparotomy and the VVF was treated with simple interrupted suture at the vaginal wall and bladder wall using a thread SAFIL 4-0. T1 group underwent the same procedure as C group and receive additional treatment of dry amniotic 1x1 cm placed between the vagina and bladder walls that was sewn at each corner using a thread SAFIL 4-0. T2 group underwent the same procedure as C group and receive...
additional treatment of dry amniotic size 1x1cm seeding with AMMSC placed between the vagina and bladder walls that was sewn at each corner using a thread SAFIL 4-0.

Seven days after the treatment, all subjects underwent another laparotomy procedure to collect sample from the VVF with the size of 1x1 cm for inflammatory cell infiltration and TNF-α evaluation using histological examination. The sample was then washed with 0.9% NaCl fluid twice before stored in the container with 10% buffer formalin for 48 hours. Hematoxilin-eosin staining was done to evaluate the inflammatory cell infiltration (neutrophil and macrophage), while immunohistochemistry staining was done to evaluate the expression of TNF-α. The number of neutrophil and macrophage cells was evaluated under the microscope and were scored using modified Klopfleisch method. Expression of TNF-α were evaluated semiquantitatively using modified Remmele-Stegner Scale based on the intensity of expression and the number of cells/tissue area positively stained.

Acquired data was analyzed using SPSS version 17.0 (SPSS, Inc., Chicago IL). Shappiro-Wilk test was used to analyze the normality of the data. Kruskall Wallis test was used to compare the bodyweight and inflammatory cell infiltration between 3 groups. Mann-whitney test was used to compare the inflammatory infiltration between 2 group. ANOVA test was used to compare the TNF-α expression between 3 groups. Post hoc LSD test was used to compare the TNF-α expression between 2 group. A p < 0.05 was considered statistically significant.

**Result**

There were 27 NZW rabbits with no dropout included in this study. Bodyweight of the rabbits used in this study ranged from 3200 to 3700 grams. After the rabbits had been randomly grouped into 3 groups, normality test showed that the bodyweight in C group, T1 group, and T2 group was normally distributed (p = 0.172; 0.296; and 0.072, respectively). There was no significant difference between bodyweight of C group, T1 group, and T2 group (3366.67 ± 173.2 gram, 3388.89 ± 145.3 gram, and 3455.56 ± 159 respectively; p = 0.461).

TNF-α expression was evaluated semiquantitatively using modified Remmele-Steiger Scale based on the intensity of expression and the number of cells/tissue area positively stained.

Acquired data was analyzed using SPSS version 17.0 (SPSS, Inc., Chicago IL). Shappiro-Wilk test was used to analyze the normality of the data. Kruskall Wallis test was used to compare the bodyweight and inflammatory cell infiltration between 3 groups. Mann-whitney test was used to compare the inflammatory infiltration between 2 group. ANOVA test was used to compare the TNF-α expression between 3 groups. Post hoc LSD test was used to compare the TNF-α expression between 2 group. A p < 0.05 was considered statistically significant.

**Table 1. TNF-α expression between groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>8.5 ± 1.6a†</td>
<td>&lt; 0.001*#</td>
</tr>
<tr>
<td>T1</td>
<td>7.1 ± 1.2b†</td>
<td>&lt; 0.001*#</td>
</tr>
<tr>
<td>T2</td>
<td>1.6 ± 1.2c†</td>
<td>&lt; 0.001*#</td>
</tr>
</tbody>
</table>

*P < 0.05 was considered statistically significant

#One-way ANOVA test was used

†LSD test was used. Values in a column with different superscripts were significantly different (P < 0.05) from each other

**Table 2. Inflammatory Cell Infiltration Between Groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Median</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>3.0 a‡</td>
<td>&lt; 0.001*#</td>
</tr>
<tr>
<td>T1</td>
<td>2.0 b‡</td>
<td>&lt; 0.001*#</td>
</tr>
<tr>
<td>T2</td>
<td>1.0 c‡</td>
<td>&lt; 0.001*#</td>
</tr>
</tbody>
</table>

* P < 0.05 was considered statistically significant

‡ Mann Whitney test was used

# Kruskall Wall test was used

†S Mann Whitney test was used.
Discussion

There are significant differences between C group, T1 group, and T2 group in this study. TNF-α expression is highest in C group, and the expression is lower in T1 group where the subjects received an additional treatment of dry amniotic. In T2 group where the subjects received an additional treatment of dry amniotic seeding with AMMSCs, the expression of TNF-α was the lowest compared to C group or T1 group. This finding showed that seeding amniotic stem cells can significantly decrease TNF-α as pro-inflammatory cytokines. Previous study that compared between the amnion and amniotic administration with seeding adipose stem cells on healing of rat skin found that in group received stem cells treatment had accelerated wound healing with minimal inflammation.

Amniotic membrane has anti-inflammatory effects and helps the wound healing process. Previous study proved its effectiveness in the cornea reconstruction and skin wound healing. Other study which compared the use of dry amniotic scaffold hyaluronic acid based on case repair tendon found a significant reduction in TNF-α expression on the seventh day. Another study found that in rabbits given mesh made from propylene compared with added amnion, the level of inflammation is significantly decreased when receive the amniotic treatment.

Amnion is part of the fetus during pregnancy which express nonclassic Human Leukocyte Antigen G (HLA-G) (class Ib antigen). HLA-G polymorphism of this type is lower than the mother’s antigen class. Because of the low immunogenicity, maternal immune system does not attack the fetus. There are two proposed mechanisms of how the amniotic membrane has a low immunogenicity. The first one is that the HLA-G plays a role in reducing the activity of lymphocytes and dendritic cell when it binds to receptors inhibitor. The second mechanism is when HLA-G recognized by CD8 T cells and activated, CD8 would bind to HLA-G and will have the immunosuppression function. This mechanism might explain why there has been no rejection to the seeding amniotic stem cell.

In our finding, there are significant differences in inflammatory cell infiltration between groups. In C group where the subjects did not receive additional treatment other than surgical treatment, the inflammatory cell infiltration was significantly higher compared to the other groups. In T1 group where the subjects receive additional treatment of dry amniotic compared to T2 group where the subject receive additional treatment of dry amniotic seeding with AMMSCs. Previous study in the case of duodenum repair showed that suture plus amnion patch compared to primary suture alone had a lower inflammatory infiltration of histopathologic score. Anti-inflammatory mechanism of amniotic believed to be a factor in reducing the response of inflammatory cells in the tissue. In addition, amnion contain growth factors and multipotent cells that help the process of angiogenesis and fibroplasia.

Mesenchymal stem cells can regulate the proliferation, activation, and as an effector for T cells, dendritic cells, macrophages, NK cells, and neutrophils. Stem cells that are affected will polarize the inflammatory environment and convert M1 into M2 macrophages that have anti-inflammatory properties. M2 macrophage phagocytic activity and the secretion of proinflammatory cytokines such as IL-12 and TNF-α is low. Factors affecting the intermediary of M2 is PGE2, TNF-α stimulated gene 6 (TSG-6), and IDO. In the acute inflammatory phase, mesenchymal stem cells protect neutrophils from apoptosis through activation of TLR3. In chronic inflammatory phase, the effect of IFN-γ reduction will reduce the migration of neutrophils to the network to prevent further damage.

Macrophages control lifespan of neutrophils to secrete receptor death ligands as Fas ligand (FasL), TNF-α and TRAIL. When FasL is secreted at low concentration, it will extend the lifespan of neutrophils and increase infiltration into the wound tissue. TNF-α delay the apoptosis of neutrophil through transcription factor NF-κβ in order to improve the tissue survival. TGF-β thought to regulate the activity and function of neutrophils. TGF-β inhibition will increase the infiltration of neutrophils in tissues. Analogous to the M1 and M2 macrophage polarization, polarize TGF-β phenotype of neutrophils. Provision of amniotic stem cells will decrease the expression of TNF-α in wound tissue, where low TNF-α will not suppress the expression of TGF-β. This is in accordance with the histopathologic evaluation of T2 groups in our study where there are decreased neutrophil infiltration compared to T1 and control.
Conlussion

Amniotic membrane could reduce TNF-α expression and inflammatory cell infiltration during the VVF repair healing process in NZW rabbit model. Seeding the membrane with AMMSC enhance the reduction effect more significantly.

Conflict of Interest: There is no conflict of interest

Source of Funding: This study is self-funded

Ethical Clearance: This study was ethically approved by Ethics Committee of Faculty of Medicine Universitas Airlangga (Ethical Clearance Number: 171/EC/KEPK/FKUA/2017) before conducting the study. All experiments were performed in accordance with relevant regulations.

References

suPAR is a Bad Omen for Chronic Kidney Disease Progression

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Abstract

The rationale for considering serum soluble-urokinase plasminogen activator receptor (s-uPAR) concentration as a potential biomarker for incipient CKD was primarily based on reports that increased levels of this receptor will serve as a circulating permeability variable that may be impaired at the start of FSGS and DN, a crucial explanation for CKD. So in early stages(1-3) of CKD, suPAR was evaluated. The aims of present paper are to investigate whether patients with CKD have distinct circulating suPAR, that could lead to a potential development of noninvasive diagnostic biomarkers of the disease. And finally using healthy subjects as a control group to determine specificity and sensitivity. 135 subjects were incorporated in this study, 30 were healthy control, with mean age 48.7±9.7years, and they were 14 males and 16 females. 105CKD patient, with mean age 50.1±10.0years, they were 54 males and 51 females. Blood samples were collected. Serum after centrifugation was isolated for estimation of suPAR by Enzyme linked immune sorbent assay, creatinine, and urea by enzymatic method. Early morning urine sample was collected to be used for determination albumin creatinine ratio in patient. suPAR level was higher in CKD patients than in healthy control, 6.179±2.221 versus 2.303±0.475, respectively; the difference was highly significant (P < 0.001). To study the potential role of suPAR in diagnosis of patients with CKD, ROC showed that the cutoff value of suPAR was > 3.5 ng / ml fold shift with sensitivity and specificity; the AUC was 0.826 (95% confidence interval: 0.690-0.920) and therefore 82.6 % precision and significance level of (P < 0.001). In conclusion, The suPAR is significantly more effective and also more comprehensive to CKD clinical assessment and can be supported with creatinine blood analysis.

Key words: CKD, soluble- urokinase plasminogen activator receptor, creatinine, albumin creatinine ratio

Introduction

Chronic kidney disease (CKD) is increasingly recognized worldwide as a major concern for public health. It is a leading cause of mortality and morbidity and a major economic burden on health care systems. It is difficult to recognize the early stages of CKD since people affected are typically asymptomatic. It often stays undiagnosed until kidney function failure is serious. CKD is distinguished by a progressive deterioration of kidney function. The most common risk factors of CKD are hypertension, diabetes, and conditions that less commonly induce CKD include glomerulonephritis. In medical practice, a strategy of checking kidney disorder is restricted to measure urinary protein secretion and estimate the glomerular filtration rate (eGFR). Proteinuria and reduction in eGFR is enormously insensitive to early infection and has restrained advantage in screening for CKD. Therefore, more sensitive biomarkers are wanted to discover at-threat sufferers in advance in the disorder process. So in early stages of CKD, suPAR novel maker was evaluated. suPAR is the uPAR receptor’s soluble form and can be determined in serum/ plasma. SuPAR is considered a biomarker of inflammation and is high in both acute and chronic diseases. Increased levels of suPAR were related to poor effects in numerous patient populations. Further, suPAR has been involved in the kidney disease pathogenesis, particularly diabetic nephropathy and focal segmental glomerulosclerosis, via intervention with migration of podocyte and apoptosis despite the fact that those findings are nonetheless beneath research, they recommend a likely increase suPAR function in kidney disorder. We examined the hypothesis that levels of serum suPAR are related to early stages (1-3) of CKD.
Material and Method

The study was conducted on 105 Iraqi CKD patients, 52 females and 49 males, The age mean of 55.6 ± SE 1.13 randomly selected from those attending the to Baghdad Teaching Hospital /Medical City form between Nov. 2018 to Mar. 2019. A well-structured questionnaire was filled for every subject and patient after full clinical examination by their consultant physicians. Patients define by using both GFR and urinary ACR. For the purpose of comparisons, 30 Iraqi control subjects comparable to CKD patients in respect to age (35-59 year) and gender (16 females and 14 males), were included in the study. Blood and urine were collected at the same visit from each subject. Before clinical examination, a permission was taken from the about the following clinical examination and biochemical tests performance.BMI,PB,eGFR. GFR was calculated using the modification of Diet in Renal Disease (MDRD),and ACR estimated from dividing the value of urine microalbumin in (mg/L) to urine Creatinine (mg/dl). Serum after centrifugation was isolated for estimation of suPAR by Enzyme linked immune sorbent assay, creatinine, and urea by enzymatic method.

Result and Discussion

The mean (± SD) values, rang, and distribution of studied groups (number and %) for the groups, age (in years), gender, body mass index, SBP, DBP, stages of diseases and duration of the disease in years of the studied groups with their significanc are shown in Table 1. Primary diagnosis of CKD has been categorised as presented by the UK Renal Registry (UK Renal Registry, 2018) by the main diseases which cause CKD. As shown in table 1 below. The CKD groups had similar etiology of CKD, includes 90 CKD patients; of whom 35 (33.3%) type 2 diabetic patients, 35(33.3%) hypertension patients and 35 (33.3%) GN patients. The mean age for CKD group was (50. 1±10.0) which was comparable to that of healthy control mean (48.7±9.7) years which was statically non-significant (P >0.05). The highest proportion of the CKD patients were found to be in the age group of more than 60 years. For older patients with diabetic, raise BMI, higher systolic and diastolic blood pressure (BP), and higher levels of hemoglobin A1c, the prevalence of CKD is higher.4 of CKD patients were males while the rest were female compared to 6 females and 14 males in the healthy controls which statically was not significant (P <0.05), highest proportion of the CKD patients 51.5% were male. The findings matched previous study (8). The development of CKD may differ depending on sex. Male patients have a significantly higher prevalence of CKD and occurrence of ESRD than female patients (9). ACR incidence and decreases in eGFR may be higher for men with diabetes than for women with diabetes(10). Chang et al (2016) concluded that maintaining blood pressure at normal levels may prevent the development of ESRD in both male and female patients. Regarding BMI, the table shows that mean BMI for CKD group was (27.21±3.65) which was comparable to that of healthy control mean (27.02±2.91) years which was statically non-significant (P >0.05). High proportion of patients (41.9%) were overweight. Patients with CKD groups clearly showed that BMI had no significant difference in comparison with controls, and no significant differences were observed in BMI between the (normal, overweight and obese) ranges as in table 1.

These results are consistent with Tian-Jong Chang et al 2018. The incidence of hypertension and DM in overweight and obese patients with CKD (all stages of CKD) was significantly higher. This clinical correlation may not reflect cause and effect; because obesity as a whole is correlated with multiple adverse sequelae of metabolic syndrome, It is consistent with CKD as well as with comorbidities like DM and hypertension(12). Obvious variation in means of systolic blood pressure between CKD and controls(127.6±20.1) and (116.3±7.7) with range (99.9-180) and (100-130) receptivity. Patients subjects showed a significant elevation in diastolic BP Hypertension affects ~30% of the general adult population and up to 90% of those with CKD(13).
Concerning the stages and duration of CKD, table 1 shows that mean ±SD was 7.4±4.9 with range from 1 to 16, highest proportions of CKD cases were mainly in stage 3 and duration more than 15 years. Elaine et al (2018) noted that the majority of patients spend a considerable amount of time in stage 3a of CKD, the presence of specific risk factors such as proteinuria diabetes, and uncontrolled systolic BP, significantly reduces the time invested at this stage. Aggressive management of such risk factors in stage 3a of CKD could be correlated with significant absolute gain (in years), and identifying patients at risk of progression at this early stage of CKD can encourage more concentrated efforts to prevent progression of CKD (For example, by avoiding non-steroidal anti-inflammatory drugs or iodine contrast), even though it’s early and mild (14).

Consider table 2 and a histogram 1 derived from suPAR concentration values representing various early stages of the CKD group. Within this distribution a suPAR levels of 5.570 is associated with the 50th percentile. In other words, 50% of the measured suPAR values are less than 5.570 as shown that the median is a strong estimate of the central direction while 50% of the controls fall below the level of 2.530 of suPAR. Moreover the percentile means below the level of 11.210 in CKD are 99% of sample fall while 99% of the controls fall below the level of 2.855 of suPAR. Serum suPAR level ranged between (1.210-2.855) and (3.450-12.340) ng/ml in healthy controls and CKD cases respectively, statistically significant differences were found between mean serum suPAR level of CKD patients and healthy controls. Biomarkers are still scarce for risk assessment in early stages of CKD patients. Recently, suPAR has been correlated with deteriorating renal function, the
risk of ESRD progression. Besides its function in activating αV β3 in podocytes, suPAR can mediate renal damage in several molecular pathways. Blocking β3 integrin with the use of a monoclonal antibody was protective in animal models of diabetic kidney disease. Therefore, suPAR can associate with several other molecules to cause podocyte dysfunction and mediate CKD progression under a wide range of conditions(15).

Table 2: Assessment mean ± SD of suPAR for CKD and healthy groups

<table>
<thead>
<tr>
<th>suPAR (ng/ml)</th>
<th>CKD</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td>6.179±2.221</td>
<td>2.303±0.475</td>
</tr>
<tr>
<td>Standard Error of Mean</td>
<td>0.217</td>
<td>0.087</td>
</tr>
<tr>
<td>Range</td>
<td>3.450-12.340</td>
<td>1.210-2.855</td>
</tr>
<tr>
<td>Percentile 05th</td>
<td>3.585</td>
<td>1.210</td>
</tr>
<tr>
<td>25th</td>
<td>4.520</td>
<td>2.110</td>
</tr>
<tr>
<td>50th (Median)</td>
<td>5.570</td>
<td>2.530</td>
</tr>
<tr>
<td>75th</td>
<td>7.800</td>
<td>2.660</td>
</tr>
<tr>
<td>95th</td>
<td>10.500</td>
<td>2.810</td>
</tr>
<tr>
<td>99th</td>
<td>11.210</td>
<td>2.855</td>
</tr>
<tr>
<td>P value (Compared to healthy control)</td>
<td>0.0001*</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 shows that the mean suPAR was higher in patients with age from (55-59) years, in males compared to females. Result was agreement with recently study. Schulz et al (2017) who stated that higher baseline levels of suPAR are linked with increased incidence of CKD and hospitalization as a result of impaired kidney function in the middle-aged cohort(16). Regarding the anthropometric measurements, mean was found to be higher in those with overweight and centrally obese. Existing evidence suggests that suPAR in the obese patients may be correlated with macrophage accumulation in adipose tissue and is strongly influenced by adiposity (17). Also in the present study the mean suPAR level was higher in obese individuals than the overweight and lean individuals.
Table 3 shows the mean (± SD) value of serum suPAR level by epidemiological variables in CKD and healthy control groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>suPAR (ng/ml)</th>
<th>CKD</th>
<th>Healthy controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Mean±SD</td>
<td>No</td>
<td>Mean±SD</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40y</td>
<td>14</td>
<td>5.417±1.555</td>
<td>3</td>
<td>1.885±0.394</td>
</tr>
<tr>
<td>40---44</td>
<td>25</td>
<td>4.984±1.044</td>
<td>11</td>
<td>2.296±0.562</td>
</tr>
<tr>
<td>45---49</td>
<td>18</td>
<td>5.442±1.598</td>
<td>3</td>
<td>2.695±0.020</td>
</tr>
<tr>
<td>50---54</td>
<td>12</td>
<td>7.136±2.296</td>
<td>4</td>
<td>2.639±0.026</td>
</tr>
<tr>
<td>55---59</td>
<td>9</td>
<td>8.238±2.075</td>
<td>4</td>
<td>2.389±0.206</td>
</tr>
<tr>
<td>=&gt;60y</td>
<td>27</td>
<td>7.061±2.743</td>
<td>5</td>
<td>1.998±0.504</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td></td>
<td>0.0001*</td>
<td></td>
<td>0.113</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>54</td>
<td>6.657±2.397</td>
<td>14</td>
<td>2.275±0.557</td>
</tr>
<tr>
<td>Female</td>
<td>51</td>
<td>5.673±1.913</td>
<td>16</td>
<td>2.328±0.408</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td></td>
<td>0.023*</td>
<td></td>
<td>0.766</td>
</tr>
<tr>
<td><strong>BMI (Kg/m²)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (18.5-24.9)</td>
<td>31</td>
<td>4.681±1.289</td>
<td>6</td>
<td>2.136±0.709</td>
</tr>
<tr>
<td>Overweight (25-29.9)</td>
<td>44</td>
<td>6.787±2.066</td>
<td>20</td>
<td>2.338±0.436</td>
</tr>
<tr>
<td>Obese (=&gt;30)</td>
<td>30</td>
<td>6.836±2.496</td>
<td>4</td>
<td>2.380±0.259</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td></td>
<td>0.0001*</td>
<td></td>
<td>0.636</td>
</tr>
<tr>
<td><strong>Stage</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I (GFR ≥90)</td>
<td>11</td>
<td>4.980±1.873</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>ACR= 10.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage II (GFR= 60-89)</td>
<td>37</td>
<td>5.808±1.513</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>ACR= 32.98</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage IIIa (GFR= 45-59)</td>
<td>33</td>
<td>6.225±2.558</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>ACR= 294.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage IIIb (GFR= 30-44)</td>
<td>24</td>
<td>7.239±2.450</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>ACR= 593.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td></td>
<td>0.019*</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Duration (years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1---4</td>
<td>36</td>
<td>5.132±1.323</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>5---9</td>
<td>32</td>
<td>6.698±2.438</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>10---14</td>
<td>21</td>
<td>6.657±2.476</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>=&gt;15y</td>
<td>16</td>
<td>6.870±2.369</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td></td>
<td>0.005*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*ANOVA test is significant at the 0.05 level of significance for difference of more than two means
*The Pearson Chi-square test is significant at the 0.05 level of significance for two means
Cancello et al found that the total amount of suPAR protein was significantly higher in obese individuals, compared to lean controls. suPAR was significantly more expressed in white adipose tissue of obese individuals, compared to lean controls\(^{(17)}\).

The mean suPAR concentrations were determined for all the different stages after classifying patients according to the proposed KDIGO classification of CKD. As shown in table 3 the suPAR concentrations increased significantly with increased albuminuria. (p<0.005). Interestingly, a total of 11 of the patients had no signs of kidney damage (i.e., eGFR >90 ml/min/1.73m\(^2\) and albuminuria <30 mg/g creatinine) together with suPAR levels > 3.5 ng/ml.

In this research, the consequence was an inverse relation of the suPAR level with the eGFR. Previous research has shown an inverse correlation between suPAR and eGFR; \(^{(18)}\) consequently, the present observation is aligned with the Wei et al results, in their analysis of two large populations, found inverse relationship between eGFR and suPAR\(^{(19)}\). Because the major suPAR fragment's molecular weight is 22kDa, which should be sufficiently low to cross through the glomerular filtration barrier, the correlation of suPAR levels with kidney function inversely seems reasonable. While a kinetic analysis will be needed to verify this hypothesis, suPAR is likely to accumulate in patients with renal disease\(^{(20)}\).

ROC analysis allowed the estimation of the utility of suPAR as the indicator of the patient’s progression. A suPAR concentration equivalent to 3.5 ng / mL has been identified as the best fit cutoff value with a sensitivity of 93 % but a precision of 91.5 % (p=0.001).That means, the test value higher than 3.5 ng/ml represents the abnormal case (CKD), whereas the value is less than 3.5 ng/ml consider healthy condition as shown in Figure2. ROC analysis further demonstrated that suPAR was a sensitive indicator for the stage of CKD, suggesting that serum suPAR could be a valuable biomarker for early diagnosis of CKD:

The comparative ROC analysis is presented in Fig 3, we took the area under the curve as a measure of assay efficiency, i.e., 0.89(P <0.001), for the suPAR assay and 0.68( P=0.032) for S.Cr assay in the case when the two assays used to predict cases with CKD differentiating them from healthy control, by this measure, the suPAR assays was clearly more valid than S.Cr, the difference statistically significant between them correspondingly when the two assays used to predict CKD progression, from the area, the suPAR assays was valid and efficient and the suPAR area seems to be slightly bigger than S.Cr.
Conclusion

The suPAR is significantly more effective and also more comprehensive to CKD clinical assessment and can be supported with creatinine blood analysis.

Conflict of Interest: Nill

Source of Funding - Self

Ethical Clearance – This study was conducted with the consent of the volunteers and without mentioning the names with the complete privacy of volunteers

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Interventions on Women Under Intimate Partner Violence: An Integrative Review

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Abstract

The present study is aimed to determine the interventions on women under intimate partner violence. This integrative review was performed by searching in international (Web of science, PubMed, Scopus Science direct, and EMBASE) and national (SID and magiran) databases were searched from inception to December 31, 2018. The keywords used included “interventions” OR “IPV” OR “intimate partner violence”. The search of studies, data extraction and screening conducted by two researcher. Of 2782 studies 30 studies included in final stage. These studies were mainly investigated the effect of the intervention type on domestic violence against women. Of these, 17 studies focused on victims of domestic violence, of which 7 focused on supporting and empowering victims of violence, and 7 were interventional studies, and the remaining 3 studies were online and computer-based interventions. Another category of interventions focused on training and empowerment of health professionals and providers (7 studies), and two studies focused on both groups of DV victims and care providers. In addition to the DV victims and care providers, 4 studies included community factors in interventions. It also seems that carrying out comprehensive and adequate interventions on women under domestic violence is needed.

Key Words: Domestic Violence; intimate partner violence; Interventions; Women; integrative review

Background

IPV is one of the most common problems in the world today, so eliminating it is one of the Sustainable Development Goals (SDGs) [1]. According the Centers for Disease Control and Prevention (CDC) the term “intimate partner violence” describes physical, sexual, or psychological harm by a current or former partner or spouse. IPV includes four types of behavior: Physical violence, Sexual violence, Stalking, and Psychological aggression [2]. According to the latest WHO statistics in 2013, the global prevalence of IPV is 30% [3]. Women with IPV are at high risk for immediate and long-term psychological depression (the most common violence-induced psychological consequence [4-8], low level of social support [4], post-traumatic stress syndrome [5,6], anxiety, sleep disorders, emotional disturbances, memory loss, poor self-esteem, fears, worries, and poor social communication [6], substance abuse, digestive disorders and chronic pain syndrome [8], more alcohol consumption, more unwanted pregnancies, and increased violence-induced abortions [7]. There has been a growing attention, at the international level, to the potential role played by health services in identifying and supporting women who have experienced violence and facilitate their referral to specialized services [6,9]. To meet the multiple complex needs that women experience with IPV in low-income and middle-income countries, the health sector needs to show a coordinated, comprehensive, and integrated response [10]. As a common worldwide health problem, preventing and combating violence against women requires interaction between different strata of society. To improve the quality of life as well as the mental health status of women with DV, gender and culture-based cultural and security interventions are also necessary [11]. To ensure their effectiveness, interventions seeking to reduce IPV and its consequences should consider the complexity of women’s experiences.
of IPV. Despite the greater recognition of IPV as a major public health problem and evidence suggesting women victims of IPV use healthcare services more frequently, little effort has been made to develop interventions aimed at reducing IPV or its consequences. A number of reviews have concluded that there is weak evidence that supports specific interventions for women victims of violence, especially interventions in health centers or those to whom health care providers can refer women [12]. Previous meta-analyses on violence interventions show that these interventions have a limited efficiency since they are all in one form and do not pay attention to the diversity of characteristics, needs, and levels of risk that distinguish IPV victims [13]. Considering the global importance of domestic violence against women and its high prevalence [14,15] and multiple IPV-induced physical and psychological consequences [4-8,16], and consequently the need for comprehensive interventions for the prevention and treatment of domestic violence victims, the present study was conducted to review the previous studies on interventions for women with domestic violence.

Materials and Method

Inclusion studies included descriptive, population-based, cohort, and case control studies that were conducted on women under IPV. Clinical trial, review, letter to editor studies, low-quality studies, studies that did not allow access to the full text version, and studies written in languages other than Persian and English were excluded from the study. The International (PubMed, Science Direct, WOS, Scopus, and Embase) and National (Scientific Information Database (SID) and Magiran) databases were searched for relevant studies without time limits in English and Persian languages from inception to 30 December 2018. The MEDLINE search strategy was adopted to search in other databases. Boolean operators (AND, OR, and NOT), Medical Subject Headings (MeSH), and related text words were used for search using the following keywords: “Interventions”, “Surveillance plan”, “intimate partner violence” and “IPV”. The last Search was conducted in 20 February, 2019. Extracted data items included: first author; year of publication; Age; Design; and intervention characteristics.

Results

A total of 2782 articles were retrieved in searched databases. Out of 2110 non-duplicated studies in the title and summary screening process, 2053 studies were excluded due to inappropriate titles. Out of 57 studies, 30 had eligibility criteria. Out of 27 excluded studies, two studies were letter to the editor, two studies were case report, 20 studies were published in Non-English and non-Persian language non from same data, and three studies did not meet the minimum quality requirements for inclusion in the study. Of the 30 studies reviewed, 17 were experimental studies (randomized controlled trial), 4 quasi-experimental studies, 5 systematic review studies, 3 mixed studies, and 1 qualitative study. Studies have mainly focused on the effect of the intervention type on IPV, of which 17 studies focused on victims of DV, of which 7 studies focused on the support and empowerment of violence victims, 7 studies on psychological interventions; and 3 studies on online and computer-based interventions. Another category of interventions (7 studies) emphasized the training and empowerment of health professionals and providers and two studies focused on both groups of DV victims and care providers. Four studies also included community factors in interventions in addition to victims and care providers [17-20]. The effects of interventions were evaluated as pre and post-intervention evaluations and often followed by follow-ups 3, 6, 9, 12, and 18-months after the intervention. Studies were carried out in various countries such as Uganda, Nepal, Canada, the United States, Mexico, the Netherlands, South Africa, Tanzania, Peru, India, Portugal, Ethiopia, England, Colombia, Denmark, Belgium, and New Zealand and encompassed diverse cultural backgrounds in relation to the DV phenomenon.

Discussion

The studies reviewed had implemented interventions in three groups focused on women victims of violence, care providers, community factors. In women-centered study group, some studies focused on empowerment and support. Fanslow’s intervention focused on raising women’s awareness of the cycle of violence, safety planning, and referral to supportive organizations such as shelters [21]. Constantino et al. carried out an intervention aimed to provide resources for women, including information about resources, time to access resources, if available, and an environment for dialogue with counselors and friends [22]. Similarly, Cripe et al. carried out an intervention to empower pregnant women under domestic violence by giving a referral card so as to use legal and social services, supportive counseling, training and guidance on safety issues [23]. Two studies
also included screening for IPV in the routine health screening, referral and supportive care, safety planning and counseling for harm reduction, and one 3-month follow-up counseling session was implemented for women subjected to domestic violence [24,25]. Findings of a systematic review carried out by Alvarez et al. who focused on interventions on women with DV have referred to interventions such as brochures, safety planning, referral, support, guidance, home visits, and group sessions for behavioral change [26]. A multi-dimensional systematic intervention carried out by Rhodes who targeted both women victims and men who committed violence, includes 3 preparation stages (social health assessment), implementation stage (social health distribution, creation of a social marketing campaign, social health distribution survey, creation of social marketing campaign, the presence of a trained family health advocate, presence an IPV advocate) and a stabilization stage (continued education of care providers, social marketing, and social health monitoring) [27].

An intervention called “DIL MIL” targeting young married women, their mothers, and spouses in India was implemented as a group session for each group and joint sessions on communication skills training, gender-based violence, family health, reflection of culture and gender roles, intergenerational communication, empowerment for change, culture, and leadership [28]. Van Parys’s intervention consisted of the following three sections: a questionnaire, a referral card at a size of a bank card, containing contact details of the IPV assistance services on one side and guidance for increasing safety behavior on the other side and a gift coupon, and two interviews [29]. Another category of women-centered interventions was based on psychological treatments along with supportive and safety measures. Kiley et al. carried out a psychological and evidence-based intervention that was carried out exclusively on any IPV-induced psychological risks with an emphasis on safety planning, and empowerment (raising awareness about violence and its cycle, risk assessment, and protection methods), taking into account a list of social resources along with the address and telephone number [30]. This model consists of four stages: 1) Start: Thinking about violence against women and HIV as issues that require health workers and community members to work together and strengthen their power to address these issues. 2) Awareness: Increased awareness about community acceptance of men’s exercise of power against women that increases violence against women and HIV. 3) Support: Support for women and men who are directly affected by these issues. 4) Action: Take actions to prevent violence against women and HIV [31]. Similar to the above intervention, a community-based study included holding sessions with local institutions and beneficiaries to examine barriers to implementing IPV interventions, as well as increasing beneficiaries’ engagement to prevent domestic violence against women, social mobilization, making women aware of gender equality, social rules and norms, raising the spouses’ level of awareness and participation and educating community representatives to engage them in changing traditional attitudes [20]. Clark et al. also conducted radio plays based on a social behavior change strategy, with interactive voice response and SMS service for listeners. They aimed to prevent IPV through three key approaches of support, social mobilization, and behavioral change (Clark et al., 2017) [19]. In his systematic review, Bouery stated that the following structural interventions are effective in preventing domestic violence: financial interventions (financing programs, support for women’s production, unconditional loans), social interventions (participatory learning, community mobilization, multimedia approaches), and economic / social interventions (gender training, livelihood education (creating the future), sexual health education, paired and gender training, cash transfers, support groups for women with gender dialogue for couples) [18].

**Conclusion**

It seems that carrying out comprehensive and adequate interventions for women under domestic violence requires a multi-dimensional perspective and integration of interventions based on the needs of women under violence, care providers, and social factors.

**Conflict of Interest:** We declare that there is no conflict of interest.

**Source of Funding:** None

**Ethical Clearance:** Taken from Shahid Beheshti University of medical sciences.

**References**


The Effectiveness Comparison of Valproic Acid 500 mg and Amitriptyline 15 mg in Reducing the Frequency of Headache Attack in Patients with Tension-Type Headache

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Abstract

Background: Tension-type headache (TTH) is the most common headache. Continuous analgesic use can develop into headache caused by drug abuse (medication overused headache); thus, the preventive therapy is necessary. Amitriptyline and valproic acid are drugs reported to reduce the frequency of headache in TTH patients.

Objective: To analyze the effectiveness of valproic acid 500 mg and amitriptyline 15 mg in reducing the frequency of headache attack in patients with tension-type headache.

Methods: The study applied a Double Blind Randomized Clinical Trial involving 50 TTH patients. The subjects were divided into two groups: amitriptyline 15 mg and valproate acid 500 mg. The period of drug administration was 6 weeks. The variables that were compared were the decreasing frequency of headache and the intensity of the pain.

Results: There was no significant difference in the decrease of headache frequency (p = 0.730) and pain intensity (p = 0.430) between the amitriptyline group and valproate acid group. However, each drug effectively decreased the frequency of headache and pain intensity in TTH patients (p = 0.000).

Conclusion: There was no significant difference between the effectiveness of valproic acid 500 mg and amitriptyline 15 mg in reducing the frequency of headache in patients with TTH.

Keywords: valproic acid, amitriptyline, pain, tension-type headache

Introduction

Tension type headache (TTH), previously called muscle contraction headache, is the most common headache where most people consider it a normal headache, compared with migraine. This is a complex disorder in which various heterogeneous mechanisms play a role. TTH often occurs daily. The prevalence of chronic TTH in one year in the general population ranges from 3% in females and 1.5% in males 1. Previous studies have suggested that the prevalence of headache is 78% in a population-based study in Denmark; however, episodic TTH (one day per month or less) is the most common that does not require special medical treatment. However, 24% to 37% experience headache several times per month, 10% experience pain per week, and 2% to 3% of the population experience chronic TTH that lasts for a longer period of time in their lives 2.

Chronic TTH is a risk factor for the occurrence of analgesic abuse that will develop into headache. Persistent headache and psychiatric comorbidity often cause this chronic TTH to be overcome. Although TTH is common, pathophysiology and mechanisms remain unclear. Pathophysiology is thought to be multifactorial, involving factors of central and peripheral nervous system as well as environmental factors 3. The vagueness

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of this pathogenesis is reflected in the variation of available prophylactic drugs. The most commonly used prophylactic drugs are tricyclic antidepressants (TCA), other classes of antidepressants and muscle relaxants, but benzodiazepines and vasodilator agents are also prescribed 4. To obtain effective treatment, it was conducted a study comparing valproic acid 500 mg and amitriptyline 15 mg in reducing the frequency of headache attacks in patients with tension-type headache5.

Method

Subjects in this study were tension type headache patients who were treated at the Neurological Outpatient Unit of Dr. Soetomo General Hospital Surabaya, Indonesia that have fulfilled the criteria of inclusion and exclusion 6. Inclusion criteria included chronic and frequent episodic tension type headache that fulfilled the criteria based on the National Consensus III Study Group Headache Perdossi 2010 and aged 18-50 years old. Exclusion criteria included secondary headache patients based on the National Consensus III of the Study Group of Headache Pain in 2010, patients with a history of allergy or contra indication of the administered drug, and pregnant women, mental illness or severe illness such as malignancy. Patients who were willing to participate in the research had to fill out the informed consent sheet 7.

The study protocol was approved by the Ethical Commission to conduct basic science/clinical research in Dr. Soetomo General Hospital Surabaya. Subsequently, the subjects were divided into two groups, the group receiving amitriptyline 15 mg and the group receiving valproic acid 500 mg. Both drugs were put into capsule packs of the same color and size; thus, the researchers and the study subjects could not distinguish the contents of the capsule. This drug was given to the patients for six weeks 8. The patients were given an explanation of the headache diary that must be filled by the patients every day during the study. After the examination, the patients were sent back with a headache diary, and medicine. The patients were given 14 capsules of medicine to drink 1x1 for two weeks either while experiencing headache or no. The patients were also given analgesic of 10 tablets and consumed only if there was a headache attack. The patients were asked to return for control, handed over a headache diary and took medication every two weeks for six weeks 9.

The collected data was processed by statistical tests using SPSS 16.0 software (SPSS, Inc., Chicago, IL). The statistical test used in this study was paired t-test and independent t-test if the data was distributed normal. If the data was distributed abnormal, it was examined by wilcoxon test continued with mann withney test. Furthermore, chi square test was conducted to know the success of treatment on each group with p <0.05 10.

Result

Table 1. The Characteristics of Research Subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Amitriptyline (%)</th>
<th>Valproic Acid</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>18 (36.00)</td>
<td>18 (36.00)</td>
<td>1.000</td>
</tr>
<tr>
<td>Female</td>
<td>7 (14.00)</td>
<td>7 (14.00)</td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JHS</td>
<td>10 (20.00)</td>
<td>9 (18.00)</td>
<td>0.840</td>
</tr>
<tr>
<td>SHS</td>
<td>9 (18.00)</td>
<td>11 (22.00)</td>
<td></td>
</tr>
<tr>
<td>University</td>
<td>6 (12.00)</td>
<td>5 (10.00)</td>
<td></td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed</td>
<td>12 (24.00)</td>
<td>9 (18.00)</td>
<td>0.390</td>
</tr>
<tr>
<td>Unemployed</td>
<td>13 (26.00)</td>
<td>16 (32.00)</td>
<td></td>
</tr>
<tr>
<td>Coffee drinkers Yes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>3 (6.00)</td>
<td>2 (4.00)</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>22 (44.00)</td>
<td>23 (46.00)</td>
<td></td>
</tr>
<tr>
<td>Stressor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2 (4.00)</td>
<td>1 (2.00)</td>
<td>1.000</td>
</tr>
<tr>
<td>No</td>
<td>23 (46.00)</td>
<td>24 (48.00)</td>
<td></td>
</tr>
</tbody>
</table>
The number of samples at the beginning of the study were 50 respondents. Most of the subjects were females of 36% in the amitriptyline group and 36% in the valproic acid group. Senior High School students were 18% in amitriptyline group and 22% in the valproic acid group. Employed subjects were 26% in the amitriptyline group and 32% in the valproic acid group. The subjects who did not consume coffee were 44% in the amitriptyline group and 46% in the valproic acid group. The subjects who did not have stressor were 46% in the amitriptyline group and 48% in the valproic acid group.

Table 2. Frequency and intensity of pain of research subjects

<table>
<thead>
<tr>
<th></th>
<th>Amitriptyline</th>
<th></th>
<th>Valproic Acid</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (Min- Max)</td>
<td>Mean±SD</td>
<td>p</td>
<td>Median (Min- Max)</td>
</tr>
<tr>
<td>TTH1</td>
<td>3.75 (1.17-750)</td>
<td>2.00 (0.00-5.66)</td>
<td>0.000</td>
<td>5.00 (1.75-7.5)</td>
</tr>
<tr>
<td>TTH2</td>
<td>6.07±1.38</td>
<td>3.17±2.18</td>
<td>0.000</td>
<td>5.54±1.08</td>
</tr>
</tbody>
</table>

The result of statistical analysis obtained the frequency of headache before and after treatment using amitriptyline with p = 0.000. In addition, valproic acid group obtained p = 0.000. The results of statistical analysis on the intensity of headache before and after taking amitriptyline obtained p = 0.000. The same condition was found in the subjects consuming valproic acid with p = 0.000.

Table 3. Frequency of pain, intensity of headache, and treatment success

<table>
<thead>
<tr>
<th></th>
<th>Amitriptyline</th>
<th></th>
<th>Valproic Acid</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>n (%)</td>
<td>Mean±SD</td>
<td>n (%)</td>
</tr>
<tr>
<td>Delta mean TTH</td>
<td>2.18±1.24</td>
<td>2.90±2.57</td>
<td>2.32±1.40</td>
<td>3.36±1.45</td>
</tr>
<tr>
<td>Delta Vas</td>
<td>13 (26.00)</td>
<td>12 (24.00)</td>
<td>11 (22.00)</td>
<td>14 (28.00)</td>
</tr>
</tbody>
</table>

*Odds ratio of treatment success = 1.37*

The success of preventive treatment was assessed by a decrease in the frequency of headache attacks at the end of the study at week six. The improvement of the frequency of headache attacks after treatment was the difference between the frequency of headache before treatment (mean TTH1) and the frequency of attacks after-treatment (mean TTH2), reported as delta mean TTH. Improvement in pain intensity was assessed by the difference between the value of pain intensity before treatment (VAS1) with pain intensity after treatment (VAS2), reported as delta VAS.

The success rate of treatment in both groups where the drug was considered effective if it successfully reduced the frequency of headache attacks as much as ≥ 50%. The treatment success rate was higher in the amitriptyline group than in the valproic acid group.
Odds Ratio obtained 1.37 with 95% confidence interval. The results of statistical analysis showed that there was no significant difference between success rate in both groups with $p = 0.570$.

**Discussion**

The effectiveness of amitriptyline as a chronic TTH preventive therapy is quite common. A randomized study compared amitriptyline with citalopram as a chronic TTH preventive therapy. They found that amitriptyline was more effective than citalopram in reducing headache attacks. Various publications also concluded that amitriptyline significantly reduces chronic TTH attacks compared with placebo.

Meanwhile, other studies have observed that amitriptyline may also reduce pain in the palpation of the pericranial muscles. But they also found that undesirable side effects were high in the amitriptyline group compared to other drugs. Randomized controlled clinical trials on TTH management with TCA conducted stress management therapy and a combination of both 187 patients. It was revealed that 78 (80%) of 97 patients receiving amitriptyline reported unwanted side effects such as dry mouth, drowsiness, weight gain, sweating, constipation, abdominal pain, nervousness, and increased appetite.

A systematic review and meta-analysis of the use of tricyclic antidepressants and headache involving 37 studies (30 studies using amitriptyline) showed that tricyclic antidepressants reduced the number of days of headache attacks in TTH patients and the number of headache attacks in migraine patients. However, side effects that arise are also quite a lot like dry mouth, drowsiness and stomach upset. The dose of amitriptyline used varies from 10 mg-150 mg per day. Because of this side-effect profile, amitriptyline is not an appropriate therapy for patients with multiple contraindications and elderly.

Several studies have examined the use of valproic acid as a preventive therapy for headache. A research studied the efficacy and safety of sodium valproate for long-term therapy in patients with chronic headaches. The study involved 642 patients undergoing treatment with sodium valproate, in which 138 patients received only sodium valproate. The results of this study received an average improvement of 47%, 50% decrease in the frequency of headache attacks in patients who obtained only sodium valproate (93 of 138 patients). Almost 75% of patients experienced a decrease in frequency of headache attacks by 50%. Drug side effects occurred in about 35% of patients but no serious side effects, and hepatotoxicity is also not found during treatment up to 6 years.

Previous research conducted an open-label trial of 30 patients using sodium divalproate at a dose of between 1000 mg and 2000 mg per day. Based on the index of weekly headache, pain-free days and dysfunctional days, general well-being rating and physicians global assessment of patients increased significantly in two-thirds of patients. In another study, the researchers consecutively studied 75 patients with intractable headache syndrome, dividing them into 3 groups of migraine frequency, transformed migraine, TTH and treating all patients with sodium divalproate 500 mg twice daily. Thirty-six patients (48%) reported a decrease in headache frequency by 50% or more.

A randomized double blind trial with placebo controlled enrolled 70 patients with chronic headaches. Twenty-nine migraine patients and 41 chronic TTH patients were divided into groups of valproic acid and placebo. Visual Analog Scale (VAS) and frequency of pain are used as evaluation. The initial dose used in this study was 500 mg per day given for 3 months. In this study, there was a decrease in the maximum pain of VAS and frequency of pain in the valproic acid group at the end of the study ($p = 0.000$). The incidence of rare, somnolent and tremor-like side effects was found in one patient, impotence occurred in one patient and hair loss occurred in one patient. This was in line with the results of our study, which also resulted in decreased frequency of pain and pain intensity (VAS) in the valproic acid group at the end of the study ($p = 0.000$).

The use of amitriptyline and valproic acid in this study is as preventive action. With the reduced frequency of headache attacks, the less the intensity of pain was experienced by the patient. This is probably due to the high levels of neurotransmitter inhibitors such as serotonin and GABA; thus, it suppresses the acute pain impulse that appears. This explains that prevention of headache attacks on TTH and efforts to find effective preventive drugs is still a challenge in medicine and this is closely related to the many factors involved in the pathogenesis of this headache. A previously published review shows that the average preventive therapy is less effective.
Conclusion

There was no significant difference between the effectiveness of valproic acid 500 mg and amitriptyline 15 mg in reducing the frequency of headache in patients with frequent episodic and chronic TTH.

Ethical Clearance: This research process involves participants in the survey using a questionnaire that was accordant with the ethical research principle based on the regulation of research ethic committee. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, nonmaleficence, and justice.

Conflict of Interest: The author reports no conflict of interest of this work.

Source of Funding: This study is done with individual funding.

References

Decrease of Epstein-Barr Virus Anti Early Antigen Imunoglobulin a Levels and Primary Tumor Size in Post-Cisplatin-Paclitaxel Chemotherapy in Nasopharyngeal Carcinoma Patients

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Abstract

Background: Nasopharyngeal carcinoma (NPC) associated with Epstein-Barr virus (EBV) chronic infection is a common head and neck malignancy in South China and Indonesia. Although radiation and chemotheraphy is the main therapy, it requires repeated and invasive biopsy for pathological evaluation. Therefore, a marker is required for screening including the level of anti early antigen immunoglobin a serology level.

Method: Pre-pots test, longitudinal cohort design. The PTV of 18 samples were examined using CT scan, while their serum EBV anti EA IgA level were examined using pre and post three series-cisplatin-paclitaxel chemotherapy ELISA.

Results: Although there was no significant changes in the level of anti EA IgA, however we found a decrease in the mean of pre-chemotherapy anti EA IgA level from 136.49 U/ml to 124.61 U/ml. There was significant changes in the VTP in pre and post-chemotherapy (p<0.05). The mean of VTP in pre-chemotherapy was 66.26 cm³ (SD-38.61 cm³), while in post-chemotherapy was 31.64 cm³ (SD-27.5 cm³). The delta mean of changes in anti EA IgA level was 11.8 U/ml and in VTP was 34.62 cm³. No correlation was found between the changes of anti EA IgA and the changes of VTP in post-chemotherapy (p>0.05). However, decreases were found in the level of EBV EA IgA and PTV in pre and post NPC patients.

Conclusion: There were decreases of serum EBV EA IgA level and PTV in pre and post-chemotherapy NPC patients.

Keywords: Immunoglobulin A, primary tumor volume, cisplatin-paclitaxel chemotherapy, nasopharyngeal carcinoma

Introduction

Nasopharyngeal carcinoma (NPC) is a rare head and neck malignancy except in South China and Southeast Asia including Indonesia. NPC incidence in South China is between 20-40 per 100,000 population per year and in Indonesia is 6.2 per 100,000 population per year or 12,000 new cases per year.¹ In Dr. Soetomo General Hospital and Dr. Cipto Mangun Kusumo General Hospital as the main hospital in Surabaya and Jakarta, Indonesia, respectively, nasopharyngeal carcinoma ranks fourth in malignancy after carcinoma of the cervix, breast and skin.²

EBV infection will be followed by the formation of specific antibodies against EBV antigens including anti viral capsid antigen (VCA), early antigen (EA), and Epstein-Barr nuclear antigen (EBNA). Increased

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levels of Immunoglobulin A (IgA) anti EA and VCA are commonly found in patients with KNF. Anti-EBV antibody levels, particularly IgA in NPC patients are higher than healthy individuals or patients with other kinds of malignant head and neck tumor, other organ tumors and even in other nasopharyngeal disorders.

Post-therapy NPC monitoring associated with EBV infection is performed with painful repeated biopsy and pathology examination. EBV serology examination can be used as screening for at-risk patients and occult primary tumors as well as to detect recurrence. A research shows that there is a significant association between serum EBV DNA levels of KNF patients with clinical staging and tumor progression. It is expected that EBV serology may replace the role of the biopsy.

Several studies have shown that the levels of IgA anti VCA and EA EBV increase with the appearance of NPC symptoms. Immunoglobulin A anti-EA is a tumor marker for the diagnosis of NPC because it exhibits high specificity compared to other tumor markers and IgA anti-EA will increase 1 - 5 years before NPC. IgA levels in pre-therapy have diagnostic and prognostic value, whereas NPC patients with higher levels of antibodies have a worse prognostic. The remaining high levels of IgA anti VCA and anti-EA after therapy are associated with poor prognostics. The increased serum immunoglobulin A (IgA) EBV serologic levels with normal histopathologic results should still be warranted for greater recurrence or higher risk of recurrence.

Serum IgA anti EA EBV serologic examination is required for post-therapy NPC evaluation. The purpose of this study was to determine the relationship between changes in IgA anti EA EBV levels in serum with primary tumor volume in post cisplatin-paclitaxel NPC patients in Dr. Soetomo General Hospital Surabaya. The results of this study are expected to be used as a basis for assessment of therapeutic response, early detection of KNF recurrence, and prognosis determination.

**Method**

This is an observational study with longitudinal cohort approach using pre-post test. The study was conducted in Department of ENT Dr. Soetomo General Hospital Surabaya in the period of August 2016 until January 2017. 24 new NPC patients were collected as the samples from a total of 25 patients that met the study criteria. Six samples were dropped-out due to changes in paclitaxel regimen, one patient due to an allergy, one patient continued the chemotherapy procedure in outside Dr. Soetomo General Hospital Surabaya, one patient refused to continue chemotherapy, two patients have undergone radiotherapy before the chemotherapy finished and one patient passed away.

The examination of serum EBV EA IgA level was conducted using. The basic data collected in this study consisted of the patients data based on age, gender and ethnicity. The examination results in the form of pre and post cisplatin-paclitaxel chemotherapy serum EBV anti EA IgA in NPC patients was assessed by Clinical Pathology Consultant. Pre and post cisplatin-paclitaxel chemotherapy primary tumor volume was assessed by radiology consultant. The data were analyzed using Wilcoxon Signed Rank Test and rho Pearson correlation test. This Research to find out the changes of serum EBV anti EA IgA and primary tumor volume (PTV).

**Results**

Table 1. The results of anti-EA IgA in post 3-series cisplatin-paclitaxel chemotherapy

<table>
<thead>
<tr>
<th>Cisplatin – paclitaxel Chemotherapy</th>
<th>Anti EA IgA level (U/ml)</th>
<th>Primary Tumor Volume (PTV) (cm3)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>136.49</td>
<td>66.26</td>
</tr>
<tr>
<td>Median</td>
<td>86.54</td>
<td>59.30</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>140.38</td>
<td>38.61</td>
</tr>
<tr>
<td><strong>Post</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>124.61</td>
<td>31.64</td>
</tr>
<tr>
<td>Median</td>
<td>93.07</td>
<td>20.15</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>127.80</td>
<td>27.55</td>
</tr>
<tr>
<td><strong>Δ(delta)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>11.88</td>
<td>34.62</td>
</tr>
<tr>
<td>Median</td>
<td>8.41</td>
<td>23.50</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>55.88</td>
<td>36.85</td>
</tr>
</tbody>
</table>
The most NPC patients were in the age group of 40-49 years old with seven patients (38.89%). The youngest age was 19 years old and the oldest was 62 years old. Distribution of NPC patients based on gender was described in patients were male (13 patients or 72.22%) and there were 5 female patients (27.78%). The comparison between male and female was 2.6:1. Distribution of NPC patients based on ethnicity were Javanese with 14 patients (77.78%) compared to Madura 4 people (22.22%).

The result of anti-EA IgA level in pre-chemotherapy was found to be 136.49 U/ml and standard intersection of 140.38 U/ml. The results of anti-EA IgA in post 3-series cisplatin-paclitaxel chemotherapy were found to average 124.61 U/ml and standard deviation of 127.80 U/ml (Table 1). Statistic test using Wilcoxon Sign Rank Test showed p value of 0.053. This indicated no significant changes in anti-EA IgA level in pre and post cisplatin-paclitaxel chemotherapy in patients with NPC (p >0.05).

The results of PTV in pre-chemotherapy cisplatin-paclitaxel showed the mean of 66.26 cm³ and standard deviation of 38.61 cm³. The results of precisplatin-paclitaxel showed the mean of 66.26 cm³ and standard deviation of 38.61 cm³. Statistic test using Wilcoxon Sign Rank Test showed p value of 0.001. The data indicated a significant difference of PTV in pre and post cisplatin-paclitaxel chemotherapy in NPC patients (p <0.05).

Changes in pre and post cisplatin-paclitaxel anti EA IgA levels showed Δ mean of 11.88 and Δ standard deviation of 55.88. Changes of PTV in pre and post-cisplatin-paclitaxel chemotherapy had Δ mean of 34.62 and Δ standard deviation of 36.85 (Table 4). Statistical test using Pearson correlation showed correlation coefficient (r) of 0.260 and p value of 0.298, suggesting insignificant correlation between the changes in anti-EA Ig A level and primary tumor volume in post cisplatin-paclitaxel chemotherapy in NPC patients.

Discussion

Distribution of NPC patients based on gender was the 13 male patients (72.22%) and 5 female patients (27.78%). The ratio between men and women was 2.5:1. Distribution of KNF patients based on gender, most patients were male (70%) with the ratio between men and women being almost the same throughout Indonesia, i.e. 2-3:1.24 The habits such as smoking increase the risk of KNF 2-6 times and so did the exposure to steam, dust and chemical gas at the workplace also increase the same risk. Formaldehyde exposure at workplace also increase the risk of NPC to 2-4 times. Increased risk also occured in workers who inhaled firewood smoke, and the risk increased 2 times in workers exposed to industrial heat and combustion products. This led to high incidence in men due to differences in living habits and occupations that cause males to have more frequent contact with carcinogens that caused NPC.2 Testosterone hormone which was dominant in male was suspected of causing immune response and tumor surveillance decrease and thus male are more prone to EBV infection and cancer.

The results of statistical analysis of changes in the levels of serum EBV anti EA IgA in pre and post-chemotherapy cisplatin-paclitaxel patients with NOC was not significant. Nevertheless, there was a mean decrease by 136.49 U/ml in pre-chemotherapy to 124.61 U/ml in post-chemotherapy (Table 1). This was incompatible with the chemotherapy mechanisms that caused humoral and cellular immune suppression. Humoral immunity expressed by B cells and assessed on the level of immunoglobulin.27 The similar mechanism in radiotherapy was given to NPC patients which often caused immunologic cell damage which resulted in decrease of cellular humoral immune response8.

Decreased serum EBV anti EA IgA levels after cisplatin-paclitaxel chemotherapy in this study might be due to abundant EBV not only in NPC tumor cells but also from activation of T cell infiltration, B lymphocytes, and epithelial cells capable of producing antigen associated with EBV.28,29 In addition, there was a difference of individual immune responses to various antigens which made antibodies as important markers were highly dependent on the host response to the viral antigen on the tumor.10 Changes in the latent cycle into lytic cycles in NPC tumor cells could occur spontaneously or induced by cisplatin chemotherapy, γ ray radiation, phorbol ester, sodium butyrate and bortezomib.30,31

Levels of EBV EA Ig A highly increased in 2 patients with NPC i.e. 19.84 U/ml in pre-therapy to 184.31 U/ml in post-therapy and 84.07 U/ml to 113.72 U/ml and 4 patients with NPC had slight increase, i.e. around ± 2.00 U/ml. The levels of EBV EA Ig A in patients with NPC in this study decreased in 12 patients. This was consistent with the study by Gu, et al.32 (2009) obtaining fluctuation of EBV antibody reactivity during therapy and steady follow-up. Fifteen of the 35 NPC patients tested for antibodies decreased after therapy but
13 patients showed small changes. Increased levels of EBV antibody after therapy were obtained in 5 patients while 2 patients that initially decreased had increase. This might be due to the different kinetic diversity of serologic EBV for each KNF patient during therapy as well as illustration of differences in radiosensitivity and immunological reactions. 

The results of the measurement of primary tumor volume (PTV) in post-chemotherapy cisplatin-paclitaxel showed a decrease of PTV mean in post-chemotherapy cisplatin-paclitaxel. The PTV change was statistically significant and therefore it was concluded that there was a significant PTV change in pre and post chemotherapy. This corresponded to the use of cisplatin-paclitaxel chemotherapy in the management of malignant tumors, i.e., to eradicate tumor cells or for locoregional control when used in conjunction with surgery or radiotherapy. Chemotherapy was used to treat macroscopic and microscopic metastases. Microscopic metastasis that was clinically invisible and deposited in the body would turn into macroscopic if not treated.

Pearson correlation test result on serum EBV anti EA IgA level changed with primary tumor volume change (PTV) in post-cisplatin-paclitaxel chemotherapy got the value $r = 0.260$ and $p = 0.298$. The results showed a non-significant relationship ($p > 0.05$). However, in general we found a decrease in EBV EA IgV levels and decreased primary tumor volume in patients with NPC who received pre and post cisplatin-paclitaxel chemotherapy. Interaction of cancer cells with lymphocytes or chemotherapy and radiotherapy that could have a significant effect on the immune system my explained it, and also the imbalance of humoral immunity and decreased cellular immunity triggered cancer progression and treatment failure. In this study changes in serum anti-EA IgA might be due to direct effects of chemotherapy or indirect effects of viral replication associated with growth of NPC tumor. The immediate effects of chemotherapy and cytostatic drugs were known to greatly damage lymphoid as an antibody producer but some studies have found that chemotherapy did not destroy all B memory cells and memory T cells even when examination did not get humoral immune response. Increased response of various antibodies to EBV protein at a higher stage of malignancy suggested viral replication associated with NPC tumor growth.

A study showed no statistically significant association between gender, age, and distant metastases with anti-VCA IgA antibodies, anti-EA IgA, anti-RGA IgG, and anti-EBNA IgA. There was a tendency to increase the levels of anti-VCA IgA and anti-EA IgA with a classification of N, but there was no association with the classification of T. This might be related to lymphocyte infiltration in NPC which significantly contributed to increase antibody to EBV antigen lytic phase.

The results of this study were consistent with a study that various chemotherapeutic drugs such as cis-platinum, fluorouracil, and taxol trigger alteration of latent cycle into lytic EBV virus infection in tumor cells. This change occurred through the protein signal kinase C δ, phosphatidylinositol 3'-kinase, and p38 stress mitogen-activated protein kinase but not caspase activation. This study was also conducted on mice containing KNF cells and found that combination of GCV therapy with 5 FU or a combination of GCV with cis-platinum being more effective in triggering KNF cell apoptosis than single therapy. Spontaneous induction of viral replication was more determined by the intracellular plasma environment than the factors that cause plasma cell differentiation. Lit replication of infected latent cell lines might be performed by induction including anti-immunoglobulin antibody (anti-IgG), activation of transforming growth factor β (TGF β), and activation of CD4 + T cells. The transition from the latent cycle to the EBV lytic cycle could be induced by DNA destruction agents such as chemotherapy (cisplatin), γ ray radicals, phorbol ester, sodium butyrate, and bortezomib.

**Conclusion**

There was a decrease in serum EBV EA IgA level and primary tumor volume n post-chemotherapy compared to in pre-chemotherapy and therefore could be used to monitor the success of post-chemotherapy medication.

**Conflict of Interest:** There is no conflict of interest.

**Source of Funding:** This study is self-funded.

**Ethical Clearance:** This study was approved by Ethical Commission of Health Research Faculty of Medicine University of Airlangga.

**References**


Correlation of Cardiorespiratory Fitness Levels with Functional Mobility Ability in Post Thrombotic Infarction Stroke Patient

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Abstract

Background: Stroke patients often experience functional ambulation difficulties and lack ability to walk independently due to the reduction cardiorespiratory fitness so that an understanding of the relationship between fitness levels cardiorespiratory with functional mobility ability is needed, especially for thrombotic infarction stroke patients.

Objectives: This study aims to assess the relationship between cardiorespiratory fitness levels with functional mobility ability in the first three months after thrombotic infarction stroke.

Method: Subjects are 38 post trombotic infarction stroke. Functional mobility is measured by the Timed Up and Go test. Subjects were asked to stand up from a chair whose seat is ± 46cm and the height of its back is 65cm, walk as far as 3 m, turn 1800, walk back and sit back. Data were analyzed and performed statistical test using SPSS 17.

Results: The study showed the average TUG test was 15.56-7.79 seconds with the longest travel time was 37.38 seconds and the fastest travel time was 8.16 seconds. The average of VO₂max was 10.62 ± 2.31mL / kg / min, with the lowest VO₂max yield of 5.78mL / kg / min and the highest was 14.54mL / kg / minute. The TUG test had a negative correlation with VO₂max the 6 minute walk test result.

Conclusion: There is a significant relationship between cardiorespiratory fitness level and functional mobility abilities in post thrombotic infarction stroke patient.

Keywords: Thrombotic Infarction Stroke, TUG, Cardiorespiratory fitness level, Functional Mobility Ability, VO₂max

Introduction

Stroke incidence approximately occurs in 15 million population per year worldwide, 5 million people died and 5 million people experiencing sequelae and permanent disability ¹-⁴. Stroke prevalence in Indonesia is 12.1 per 1,000 population ⁵. The number of stroke incidences continues to increase along with rising risk factors and elderly population and can affect quality of life ⁶ that leads to appropriate treatment ⁷,⁸. Motor impairment is the main cause of disability and limited mobility in individual with stroke ⁹. Mobility recovery is the main purpose of stroke patient during rehabilitation since it serves as the key factor to be independent in daily activities ¹⁰.

Stroke patient commonly has functional ambulation difficulties and reduced ability to walk independently due to decreased cardiorespiratory fitness, changes in neuromotor control and increased energy required

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for daily activities. Other research pointed out that improved Timed Up and Go (TUG) score was obtained in the first week until 3 months after stroke, while there was no significant change after those periods, and the patterns of mobility recovery differed by age groups. The age group above 80 suffer from decreased mobility in the third until the twelfth month after stroke, while mobility in other age groups tends to be stable.

Cardiorespiratory fitness decreases significantly in post-stroke patients. It brings negative effects to walking speed, endurance and performance in daily activities and stroke patient independence. Other study obtained minimum VO$_{2\text{max}}$ (peak oxygen consumption) for age group of 85 to live independently was 17.7 mL/kg/minute for male and 15.4 mL/kg/minute for female. Minimum VO$_{2\text{max}}$ in order to perform daily activities is 15 mL/kg/minute. MacKay-Lyons and Makrides, in their study on assessing VO$_{2\text{max}}$ in post-stroke patients after 1 month with body weight supported female, found average VO$_{2\text{max}}$ was 14.4±5.1mL/kg/minute or around 60% of prediction value based on age and sex.

Stroke patient has low cardiorespiratory fitness. Average VO$_{2\text{max}}$ is an indicator of average aerobic capacity from 11.7±3.7 mL/kg/minute to 17.3±7.0 mL/kg/minute or around 50-60% of normal value based on age and sex. Low training capacity is a result of increased energy usage to walk due to paretic deficits. These deficits also cause low social participation and life quality. The study is then aimed to investigate the correlation between cardiorespiratory fitness level and functional mobility ability in the first three months after thrombotic infarct stroke, factors affecting functional mobility ability and cardiorespiratory fitness level. Previous study found a correlation between TUG score and distance achieved in 6-minute walking test.

**Method**

An observational analytic study was carried out at Medical Rehabilitation Unit of Dr. Soetomo Teaching Hospital, Surabaya, Indonesia from June 2015 using cross sectional study design. The samples were taken using consecutive sampling after fulfilling the inclusion criteria: post-thrombotic infarct stroke patients aged 21-70 (first stroke attack or recurrent attack) with unilateral or bilateral hemiparesis (leg muscle strength ≥3), onset ≥2 weeks until 3 months after thrombotic infarct stroke, subjects could walk 10 meters without aid, non-assistant, could comply with simple orders and signed informed consent. The study protocol was approved by the ethics committees of Dr. Soetomo Teaching Hospital (Surabaya, Indonesia). 38 eligible subjects’ functional mobility ability were measured using TUG test and 6-minute cardiorespiratory fitness test. The data were analyzed using SPSS 17.0 (SPSS, Inc., Chicago, IL).

**Results**

**Subjects’ characteristics**

There were 38 subjects, consisting of 26 males and 12 females. The youngest and oldest subjects were 39 and 69 years old, respectively, with subjects’ average age was 55.13±8.29. The subjects’ average body mass index (BMI) was 24.25±4.05 kg/m$^2$. The subjects’ average stroke onset was 6.60±3.20 weeks. There were 32 subjects (84.2%) with first stroke attack, 16 subjects with right right-sided hemiparesis and 22 subjects with left-side hemiparesis. There were 2 subjects used walking aids. There were 16 subjects with diabetes mellitus history, 36 with hypertension history, 11 with smoking history and 1 with alcoholic history. All subjects had lower extremity muscle strength ≥3. Male subjects’ muscle motor strength of paretic lower limb was better than female subjects after enhancement. 33 subjects (86.8%) were post-stroke patients who had first visitation to Medical Rehabilitation Unit of Dr. Soetomo Teaching Hospital, Surabaya, Indonesia and had not received rehabilitation therapy, while the rest 5 subjects (13.1%) had more than one visitations and received rehabilitation therapy.

**Results of TUG and 6-minute walking tests**

The results of functional mobility test using TUG test in 38 subjects showed average TUG time was 15.56±7.69 seconds, with longest and shortest TUG time were 37.38 seconds and 8.16 seconds, respectively (Table 1).

<table>
<thead>
<tr>
<th>Variabel</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>Range value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TUG</td>
<td>38</td>
<td>15.56</td>
<td>7.69</td>
<td>8.16-37.38</td>
</tr>
<tr>
<td>VO2max</td>
<td>38</td>
<td>10.62</td>
<td>2.31</td>
<td>5.78-14.54</td>
</tr>
</tbody>
</table>

Table 2 showed mean rank of TUG time of all variables (sex, diabetes mellitus, smoking history,
Table 2. TUG test on variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Mean rank TUG</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>M 26</td>
<td>16.96</td>
<td>p = 0.038</td>
</tr>
<tr>
<td></td>
<td>F 12</td>
<td>25.00</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>Yes 16</td>
<td>17.63</td>
<td>p = 0.375</td>
</tr>
<tr>
<td></td>
<td>No 22</td>
<td>20.86</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>Yes 11</td>
<td>18.55</td>
<td>p = 0.735</td>
</tr>
<tr>
<td></td>
<td>No 27</td>
<td>19.89</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>Yes 36</td>
<td>20.14</td>
<td>p = 0.133</td>
</tr>
<tr>
<td></td>
<td>No 2</td>
<td>8.00</td>
<td></td>
</tr>
<tr>
<td>Hemiparesis</td>
<td>Right 16</td>
<td>14.5</td>
<td>p = 0.906</td>
</tr>
<tr>
<td></td>
<td>Left 22</td>
<td>16.3</td>
<td></td>
</tr>
</tbody>
</table>

The results of cardiorespiratory fitness assessment using 6-minute walking test showed the $\text{VO}_2\text{max}$ were 14.54mL/kg/minute and 5.78mL/kg/minute, respectively. Table 3 showed average $\text{VO}_2\text{max}$ of all variables (sex, diabetes mellitus, smoking history, hypertension history and hemiparesis).

Table 3. $\text{VO}_2\text{max}$ of all variables

<table>
<thead>
<tr>
<th>VO2max variables</th>
<th>n</th>
<th>Mean±SD</th>
<th>SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>M 26</td>
<td>11.32</td>
<td>1.91</td>
<td>p = 0.004</td>
</tr>
<tr>
<td></td>
<td>F 12</td>
<td>9.10</td>
<td>2.42</td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>Yes 16</td>
<td>10.64</td>
<td>2.73</td>
<td>p = 0.975</td>
</tr>
<tr>
<td></td>
<td>No 22</td>
<td>10.61</td>
<td>2.01</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>Yes 11</td>
<td>10.64</td>
<td>2.42</td>
<td>p = 0.970</td>
</tr>
<tr>
<td></td>
<td>No 27</td>
<td>10.61</td>
<td>2.31</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>Yes 36</td>
<td>10.54</td>
<td>2.34</td>
<td>p = 0.343</td>
</tr>
<tr>
<td></td>
<td>No 2</td>
<td>12.15</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>Hemiparesis</td>
<td>Right 16</td>
<td>10.79</td>
<td>2.16</td>
<td>p = 0.702</td>
</tr>
<tr>
<td></td>
<td>Left 22</td>
<td>10.50</td>
<td>2.45</td>
<td></td>
</tr>
</tbody>
</table>

Association between cardiorespiratory fitness and functional mobility ability

Spearman’s correlation test showed a negative correlation between TUG time and $\text{VO}_2\text{max}$: TUG time correlated with age ($r = 0.387, p = 0.016$), while it had no correlation with onset ($r = 0.106, p = 0.525$) and BMI ($r = 0.183, p = 0.270$). $\text{VO}_2\text{max}$ did not correlate with age ($r = -0.168, p = 0.313$), onset ($r = -0.277, p = 0.092$) and BMI ($r = 0.158, p = 0.343$; Table 5).
**Table 4. Correlation between TUG test and VO2\textsubscript{max}**

<table>
<thead>
<tr>
<th>VO2max</th>
<th>TUG</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>-0.803</td>
</tr>
<tr>
<td>p</td>
<td>0.0001*</td>
</tr>
</tbody>
</table>

r: correlation coefficient; *significant correlation in p <0.01 (2-tailed)

**Table 5. Correlation between TUG test and age, onset and BMI**

<table>
<thead>
<tr>
<th>Variable</th>
<th>TUG</th>
<th>VO2max</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>P</td>
</tr>
<tr>
<td>Age</td>
<td>0.387</td>
<td>0.016**</td>
</tr>
<tr>
<td></td>
<td>-0.168</td>
<td>0.313</td>
</tr>
<tr>
<td>Onset</td>
<td>0.106</td>
<td>0.525</td>
</tr>
<tr>
<td></td>
<td>-0.277</td>
<td>0.092</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.183</td>
<td>0.270</td>
</tr>
<tr>
<td></td>
<td>0.158</td>
<td>0.343</td>
</tr>
</tbody>
</table>

r: correlation coefficient; **significant correlation in p <0.05 (2-tailed)

**Discussion**

The study found that there is a correlation between cardiorespiratory fitness level and functional mobility ability in post-thrombotic infarct stroke patients. The higher the VO2\textsubscript{max} in 6-minute walking test, the lower the TUG score. Cardiorespiratory fitness decreased, VO2\textsubscript{max} <25.2 ml/kg/minute is associated with increased stroke risk and ischemic stroke, and this is a modifiable risk factor that requires training intervention for increased cardiorespiratory fitness \textsuperscript{17}. The average TUG time in subjects with average stroke onset of 6.60±3.20 weeks was 15.56±7.69 seconds, faster than the finding of Persson et al. They found TUG score of 17.0±11.0 seconds in the first-week test, slower than three-month evaluation (14.5±10.0 seconds) \textsuperscript{12}. The VO2\textsubscript{max} was measured using training test as a gold standard to assess cardiorespiratory fitness. Stroke patients have limitations in performing training test due to decreased strength and sensory impairment \textsuperscript{11}. Six-minute walking test is a submaximal test since patients determine their own walking speed. The test is performed in 100-feet track or around 30 meters in accordance with American Thoracic Society (ATS) guidelines \textsuperscript{20}. The study used 10-meter track since subjects in the study were subacute post-stroke patients who had not performed stress test. Therefore, emergency condition could be easily detected, and patients could be immediately treated. Pohl et al. in their study used 10-meter track for initial screening before conducting 6-minute walking test \textsuperscript{21}.

The average VO2\textsubscript{max} was 10.62±2.31 ml/kg/minute, a little bit lower than the finding of Tang et al. (12.3±3.1 ml/kg/minute) \textsuperscript{11}. Pohl et al. in their study in 72 subjects found average distance obtained in 6-minute walking test was 215.8±91.6 meters in 73.3±26.8 days of onset \textsuperscript{21}, a little bit higher than the result found in the study (206±78.6 meters). Normal distance obtained in the 6-minute walking test was 593±57 meters for female and 638±44 meters for male \textsuperscript{22}. The study found a slightly lower distance than Pohl et al. since subjects in their study had received training program, either strength, balance or endurance in lower and upper limbs for 12 weeks \textsuperscript{16}. On the other hand, 86.8% of our subjects were post-stroke patients who first visited medical rehabilitation unit. Pohl et al. used 30-meter track, while we used 10-meter track that required a lot of time for turning.

Cardiorespiratory fitness varies from age, sex, physical activity level, body composition and chronic or disability presence \textsuperscript{14}. In this study, VO2\textsubscript{max} obtained from 6-minute walking test was not significantly affected by age, stroke onset, hemiparesis and BMI. Subjects were relatively young (55.13±8.29), hence there was no decreased muscle mass and strength that subsequently shortened the distance. All subjects were in subacute phase. Our findings were not affected by BMI since 47.4% of all subjects had normal BMI. Subjects with obesity had a shorter distance. Nevertheless, VO2\textsubscript{max} was
significantly different by sex since male had a longer distance compared to female. Male was higher than female, therefore they had a wider stride length when walking. VO2max was significantly different due to hypertension risk factor, DM and smoking habit. In this study, blood pressure in subjects with hypertension history was controlled by drugs. Subjects in this study were dominated by non-DM patients (57.9%) and non-smokers (71.1%). Dourado et al. found arterial hypertension and smoking significantly affected distance during 6-minute walking test. The study found around 40% of post-stroke cardiorespiratory fitness decreased compared to normal people.

The study found significant TUG time and age differences between male and female subjects (p = 0.038 and p = 0.016, respectively). It might be due to male subjects (68.4%) were more than female subjects (31.6%), and male’s lower limb motoric improvement was better than female’s. Subjects aged <60 were 24 (63.2%). There was no decreased muscle mass, muscle strength and coordination in this age group. The study found no significant TUG time difference based on onset, BMI, hemiparesis side, DM history, hypertension and smoking. TUG score in the previous studies were affected by muscle strength, balance, coordination, spasticity and postural control anticipation. Ng et al. found a negative correlation between walking speed and TUG score at a comfortable pace, even though TUG test is a series of movements and commands requiring balance control of muscle strength and coordination.

In this study, post-stroke patient’s TUG time was longer than healthy subject. It indicated decreased functional mobility ability in post-stroke patients, in which subjects are still independently capable of main transfer with or without aids, but are at risk of failing. Distance in 6-minute walking test is used to calculate VO2max as it reflects a person’s cardiorespiratory fitness level. In this study, VO2max was negatively correlated with TUG time. The longer the distance, the larger the VO2max and it shows better cardiorespiratory fitness. The shorter the TUG time, the better the mobility status. Therefore, the higher the VO2max, the shorter the TUG time. It indicates that subject with better cardiorespiratory fitness level has better functional mobility.

**Conclusion**

The study showed a significant correlation between cardiorespiratory fitness level and functional mobility ability in post-thrombotic infarct stroke patients. The higher the VO2max in 6-minute walking test, the lower the TUG score.

**Ethical Clearance:** The study has been approved by ethical committee in Dr. Soetomo Teaching Hospital Surabaya, Indonesia.

**Conflict of Interest:** The author reports no conflict of interest of this work.

**Source of Funding:** This study is done with individual funding

**References**

9. Faria CD, Teixeira-Salmela LF, Silva EB, Nadeau S. Expanded timed up and go test with subjects


The Early Detection in Gestational Diabetes Mellitus at Indonesia Primary Health Care

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1School of Midwifery, Faculty of Medicine, Universitas Airlangga, 2 Department of Obstetric Gynecology, Faculty of Medicine, Universitas Airlangga

Abstract

Background: Gestational diabetes mellitus (GDM) is diabetes that is first diagnosed during pregnancy. Uncontrolled GDM or late found can increase mortality for both mother and baby. Not only bad medical conditions, but also it can decline the community productivity, affect socio-economic and increasing financial health. Early detection of GDM is crucial to be implemented by providers, especially in the primary health care.

Objective: to determine the framework of early detection in gestational diabetes mellitus at primary health care

Methods: The method used is a literature review, which was published in 2008-2018. The literature were collected using some keywords such as “detection”, “gestational diabetes mellitus”, and “primary health care” using PubMed and another electronic journal website. Furthermore, the findings were filtered based on our inclusion criteria and analyzed qualitatively.

Results: Early detection of GDM in primary health care with risk factor assessment (age of pregnant women ≥ 35 years, some of bad history namely GDM in previous pregnancies, anti-diabetic drug use, diabetes mellitus in the family, congenital of infants, IUFD, preeclampsia, macrosomia, blood pressure examination, calculation of body mass index and random blood sugar examination). Furthermore, early detection of GDM is carried out by examining blood glucose accompanied by the provision of appropriate glucose solution interventions (OGCT and OGTT).

Conclusion: primary health care can take more action in early detection of GDM through quality antenatal care, which can be done by applying the framework of early detection in gestational diabetes mellitus.

Keywords: Early detection, gestational diabetes mellitus, primary health care

Introduction

GDM is a glucose tolerance disorder that was first discovered during pregnancy, this condition occurs in a woman who has never been diagnosed with diabetes then shows high glucose levels during pregnancy1. GDM begins to be diagnosed at gestational age above 20 weeks2. The prevalence of GDM in Indonesia is around 14% of the total number of pregnancies, while the number of undiagnosed or diagnosed GDMs is around 10-25% of the total cases handled, this number is certainly quite large and needs to be better managed. Uncontrolled diabetes mellitus during pregnancy can increase mortality and morbidity for both mother and baby. Abortion can occur in the mother, preeclampsia, polyhydramnios, labor trauma and other complications such as diabetic retinopathy, diabetic nephropathy. In

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infants can occur cardiac abnormalities, abnormalities of the central nervous system, premature, macrosomia, brachial nerve damage, respiratory distress syndrome, jaundice, hypoglycemia, hypocalcemia and fetal death.

At the long-term risks accepted are obesity, impaired permanent glucose tolerance (DM type 2) and low intelligence whether there are mothers or babies born. Antenatal care is one of the interventions recommended to prevent maternal and infant mortality and morbidity worldwide, including in the effort to detect and treat high risk cases. Early diagnosis and detection are the key to successful management of GDM in order to achieve improved quality of life for mothers and infants.

The implementation of antenatal care in health facilities so far refers to the minimum standard elements of 10T antenatal care, namely (1) weight measurement and height measurement (2) blood pressure measurement (3) measurement of Upper Arm Circumference (LILA) (4) height measurement uterine crest (fundus uteri) (5) determination of tetanus immunization status and administration of tetanus toxoid immunization according to immunization status (6) Administration of blood tablets at least 90 tablets during pregnancy (7) Determination of fetal presentation and fetal heart rate (FHR) (8) Implementation conversation (interpersonal communication and counseling, including family planning) (9) Simple laboratory test services, minimal blood hemoglobin (Hb) test, urine protein examination and blood type examination (if it has never been done before) (10) Case management.

In the integrated ANC program, every pregnant woman receives at least one more comprehensive service, one of which is for laboratory examinations. However, the implementation of existing laboratory tests is not effective enough for early detection of GDM. In many of the findings of the integrated ANC laboratory results, the components examined focus on hemoglobin, blood group, hepatitis B, sexual transmitted illness (HIV and syphilis), urine protein and urine reduction. On examination of urine reduction results positive or negative. When linked to the gold standard for early detection of GDM (blood glucose testing by providing glucose solution interventions to produce results in the form of numbers), then the routine antenatal implementation has not been effective enough and has not been focused on diagnosing GDM. The routine antenatal examination refers more to the efforts of early detection of preeclampsia, anemia, sexually transmitted diseases, risk of bleeding and others. Based on the description above, the aim of this study is to determine the framework of early detection in gestational diabetes mellitus at primary health care.

**Method**

The method used is a literature review through an electronic search of published literature in 2008-2018. The literatures are available in soft copies; such as PDF journals and direct reading from online journal articles scientifically proven.

The literature were collected using some keywords such as “detection”, “gestational diabetes mellitus”, and “primary health care” using PubMed and another electronic journal website. Furthermore, the findings were filtered based on our inclusion criteria and analyzed qualitatively.

The inclusion criteria of the studies consists of: (1) the definition of gestational diabetes mellitus (2) the study explained the risk factor and the management of gestational diabetes mellitus (3) the studies showed the outcome of implementation the management of gestational diabetes mellitus (4) the sampling of studies, data collection and analysis are clearly described and correlating to the research questions (5) the studies included in the review were restricted to English and Indonesia languages. The result of the studies was explained qualitatively provided in the result and discussion section.

**Result and Discussion**

Based on the regulation of the Minister of Health of the Republic of Indonesia in 2014s, routine laboratory tests must be performed on every pregnant woman, namely blood type, blood hemoglobin, and specific examination of endemic/epidemic areas (malaria, HIV, etc.). While a special laboratory examination is another laboratory examination carried out on indications. Pregnant women suspected of having diabetes mellitus should have a blood sugar check during their pregnancy at least once in the first trimester, once in the second trimester, and once in the third trimester.

Gestational Diabetes Mellitus is diabetes that first arises (first onset) or is diagnosed (first recognition) during pregnancy. More than half of women with GDM eventually develop overt diabetes within the next 20 years, and there is increasing evidence of long-term
complications including obesity and diabetes in offspring caused by GDM. At more than 26 weeks’ gestation, the body produces several hormones, such as estrogen, progesterone, cortisol and HPL (Human Placental Lactogen) which have insulin resistance effects. The function of this hormonal effect is to increase nutrition and sugar in blood circulation so that it helps the growth of the fetus. As compensation, the body produces more insulin. GDM occurs when pregnant women cannot produce enough insulin or body cells are more resistant to insulin. 

GDM has no specific signs and symptoms. Pregnant women with GDM almost never give a complaint. This disease is considered mild, without any real symptoms but has a major influence on maternal and fetal morbidity both short and long term, so early detection is needed to improve the well-being of the mother and fetus both during pregnancy and after pregnancy.

Early detection of GDM is done universally in all pregnant women, not elective screening. This means conducting an examination without looking at risk factors. If only those who have risk factors are examined, then almost 50% of GDM cases will pass undiagnosed. But in the study of Trauffer (2008) recommends screening or early detection at high risk because at low risk only 0.9% suffer from GDM, at high risk around 4-7%. However, the gold standard set is for all pregnant women aged 24-28 weeks once with a 50 gram load and if the next 1 hour plasma glucose is more or equal to 130 or 140 followed by a 100 gram TTGO.

In addition to examining glucose tolerance, another screening for GDM is the assessment of risk factors. Assessments that need to be carried out include:

a. Pregnant women age ≥ 35 years

Pregnancy at too old age is included in the criteria of high risk pregnancy, death and maternal complications increased 9-fold in pregnant women aged ≥35 years. The incidence of GDM tends to be high in old age which is expected due to the influence of the aging process and damage to vessel endothelial progressive blood.

b. History of diabetes GDM in a previous pregnancy

The risk will increase in the history of pregnant women who have been exposed to GDM in previous pregnancies.

c. History of diabetes mellitus/ use of OAD/ special diet

In the history taking, there is a history of diabetes mellitus that is being suffered by the mother or the use of certain diets or the use of anti-diabetic drugs (OAD) or routine use of insulin can lead to a diagnosis of GDM so that further referral can be made immediately.

d. Family history of diabetes mellitus

Diabetes mellitus tends to be inherited or inherited, and is not transmitted. Genetic factors provide a greater chance of suffering from diabetes mellitus compared to family members who do not suffer from diabetes mellitus. If there are parents or siblings who suffer from diabetes mellitus, then the person has a 40% risk of suffering from diabetes mellitus.

e. History of congenital defects

The incidence of major fetal malformations increased by 5% in women with diabetes. Lack of diabetes control during pregnancy increases malformations in the fetus. One of them is heart defects is that oxidative stress triggered by hyperglycemia inhibits the migration of heart-filled cysts. Women who exercise glucose control in preconception care can reduce the risk of congenital anomalies.

f. History of unborn fetus (IUFD)

This incident is related to fetal metabolic disorders. Ketoacidosis can cause fetal death. In addition, mothers with GDM can find a placenta experiencing hydrops due to chorion villus edema that is triggered by osmotic pressure which causes disruption of oxygen delivery to the fetus.

g. History of preeclampsia

The incidence of preeclampsia has a relationship with glucose control during pregnancy especially at HbA1c levels.
h. History of childbirth (> 4000 gram)

The incidence of macrosomia increases significantly if the mother’s glucose concentration exceeds 130 mg/dl. The distribution of birth weight of infants of diabetic mellitus mothers always tends to be higher than that of infants of normal pregnancy.

i. Blood pressure check: hypertension results (≥ 140/90 mmHg)

Pregnant women with diabetes mellitus can trigger hypertension and most often force labor less than 8 months17.

j. Calculation of Body Mass Index (BMI)

BMI calculation results are said to be obese if ≥30 kg/m2. Obese women have a 1.7 times higher risk of giving birth to macrosomia babies compared to women who have normal weight Gestational diabetes mellitus is found to be higher in women with a higher BMI and higher body weight before pregnancy. This shows that many groups of mothers giving birth to macrosomia have risk factors for giving birth to macrosomia babies and suffering from gestational diabetes mellitus17.

k. Random blood sugar (GDA) check

The results of blood sugar can indicate the condition of glucose intolerance. The normal value for random or temporal blood sugar examination results is <200 mg/dl. This finding can be confirmed by the discovery of the classic trias of diabetes (polyuria, polydipsia, polyphagia)17.

In the framework of early detection of gestational diabetes mellitus above starting from pregnant women making the first ANC visit to primary health care, a risk factor assessment that increases the incidence of GDM is carried out. Risk factor assessments are carried out for all visiting pregnant women, regardless of gestational age. If a midwife or other health worker discovers one of the risk factors above, the midwife needs to take further management, which is to make a referral or collaborate with other health workers who have the authority to provide care according to the needs of the pregnant woman. If the risk factor data is not obtained in the assessment, pregnant women will be given routine ANC services according to standards by the midwife.

Furthermore, at 24-28 weeks of pregnancy, midwives will conduct an OGCT (Oral Glucose Challenge Test) without prior fasting preparations. In this examination, pregnant women will be given a 50 gram glucose solution. Furthermore, after 1 hour of giving glucose solution, pregnant women will be tested for blood sugar. If the results of the examination >135 mg/dl, the mother will be advised for the next examination, namely TTGO / OGTT (Oral Glucose Tolerance Test). And if the result is <135mg/dl, pregnant women will be scheduled for routine ANC according to the schedule.

At the TTGO / OGTT (Oral Glucose Tolerance Test), pregnant women are asked to fast for 10 hours. Furthermore, the mother will be examined for fasting blood sugar (positive results, if: ≥ 95mg/dl). After checking fasting blood sugar, pregnant women are given 100 gram glucose solution. Furthermore blood sugar will be checked after administration of 100 gram glucose solution at 1 hour after administration (positive result, if: ≥ 180mg/dl), at 2 hours after administration (positive result, if: ≥ 155mg/dl) and 3 hours after administration (a positive result, if: ≥ 140mg/dl). The diagnosis of GDM is made if at least 2 of the TTGO / OGTT results are positive. If only 1 positive test results are found, then antenatal care is treated the same as GDM. If the five blood sugar tests in TTGO / OGTT are negative, pregnant women will be scheduled for routine ANC according to schedule.

The framework is expected to be a reference source for primary level health facilities in providing quality antenatal services, especially in the early detection of GDM without reducing elements or antenatal service standards set by the government (ANC 10 T program and integrated ANC).

**Conclusion**

Improving the quality of antenatal services, especially in the early detection of GDM, can be done in primary health facilities through implementing an early framework for GDM detection based on screening of risk factors and further examination management.

**Conflict of Interest:** There is no conflict of interest.

**Source of Funding:** This research was funded by author.

**Ethical Clearance:** This study has been through a review and has been declared eligible ethics from the Faculty of Medicine, Universitas Airlangga, Surabaya Indonesia.
Reference


Observation of Oral Contrast Media Filling Into Lumen Appendix

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Abstract

Background: CT scan has become the imaging modality of choice in diagnosing appendicitis in unusual cases, replacing appendicogram with very little reliability. However, there is still an appendicogram demand in particular hospital in Indonesia.

Objectives: This study aimed to obtain information on lumen appendix filling pattern with multi slice Multislice computed tomography (MSCT) scan using oral contrast media in patients without clinical appendicitis. Thus, it is able to provide additional information in the appendicogram interpretation.

Method: This study was descriptive observational to 30 subjects selected by consecutive from April to June 2012. Subjects who underwent an abdominal MSCT scan using oral contrast media were analyzed descriptively by sex, age and lumen appendix filling pattern.

Results: 30% of patients were men and women by 70%. The most common age group is 51-60 years (50%). Appendix of non-filling of 56.6%, partial filling of 43.3%, and no full filling appendix was obtained.

Conclusion: Appendix filling as an indicator of the presence of appendicitis was not reliable in an abdominal MSCT scan using oral water soluble contrast, thus it needs to be considered when interpreting the appendicogram results/

Keywords: MSCT, oral contrast, appendix without clinical appendicitis

Introduction

The appendix is a dead end tubular structure that is an out pouching of the cecal tip on the posteromedial side 1. Appendicitis is one of the most common diseases in the emergency department and requires immediate surgical intervention. Typical cases have been diagnosed clinically and laboratories. Whereas, not typical cases especially in pediatric patients, parents and young women were required radiological assistance in making the diagnosis 2. Immediate and precise appendicitis treatment will reduce the risk of morbidity and mortality due to perforation and sepsis inflicted. There are various modalities in appendix imaging. Prior to the 1980s, barium appendix was still the main radiological method used for the diagnosis of acute appendicitis 3, however this modality was considered inaccurate. After 1990, CT scan was accepted as the primary imaging technology 4–6 for acute appendicitis due to visualization capabilities of the appendix, intestines and surrounding tissues 7.

Prior to 1997, surgeons agreed that 20% of appendectomy negative was still accepted because of high mortality in appendicitis coulson, however, it was unused any longer 8. Improvement in imaging technology resulted in CT scans capability to decrease negative appendectomy up to 3% 9. Therefore, Another result recommends CT scan as the diagnostic modality of choice for adult, pregnant and children. Appendix barium examination (appendicogram) is still obtained at Dr. Soetomo Teaching Hospital Surabaya. Therefore,
that is encouraging researchers to reexamine the pattern of oral contrast media fillings into the appendix lumen in patients without clinical appendicitis at Dr. Soetomo Teaching Hospital Surabaya. Observations of oral contrast media filling patterns into the appendix lumen in patients without clinical appendicitis were performed by using dilute oral water-soluble contrast and observed with Multislice Computed Tomography (MSCT) 16-slice abdominal scan. There have been no previous similar studies. Therefore, This study aimed to obtain information on lumen appendix filling pattern with multi slice Multislice Computed Tomography (MSCT) scan using oral contrast media in patients without clinical appendicitis. Thus, it is able to provide additional information in the appendicogram interpretation

**Method**

The subjects of the study were group of patients with MSCT 16 slice abdominal scans examination using oral contrast media at Radiology Unit of Integrated Diagnostic Center Building in Dr. Soetomo Teaching Hospital Surabaya from April to June 2012. The subjects were sampled using consecutive sampling that fulfilled the inclusion criteria (Patients with abdominal MSCT examinations using oral contrast media, oral contrast medium had reached cecum or ileocecal junction) and exclusion criteria (patients of post-appendectomy, patients with appendicitis (inflammatory or tumor), oral contrast media did not reach the cecum). Moreover, the subjects had signed informed consent.

This study was a descriptive research with the observational method to determine the pattern of appendix lumen filling in patients without clinical appendicitis that given oral contrast media in CT scan unit of radiology section at Dr. Soetomo Teaching Hospital Surabaya with a total sample of 30 subjects. Subjects who had fulfilled the inclusion and exclusion criteria consumed oral contrast media water soluble urography of 76% by 10 ml that diluted with 1200 ml of water, since 6-8 hours before the examination until prior to the MSCT abdominal examination. Contrast water soluble media was easier to fill the narrow lumen than barium.

**Results**

**Characteristics of Subjects**

We obtained 30 subjects consisting of 9 men (30%) and 21 women (70%). This study obtained the distribution of the most common age group; 51-60 by 15 subjects (50%), followed by the age group of 31-40 as many as 5 subjects (16.6%), age group of 11-20 years by 4 subjects (13.3%), and age group of 21-30, 41-50, and 61-70 each consisted by 2 subjects (6.6%). The observations of the 30 normal appendices showed that nothing was filled with full oral contrast media. The most common appendix was non-filled by 17 subjects (56.6%) with the distribution based on the age of the subjects. The remaining 13 subjects (43.3%) experienced partial filling with the distribution based on the age of the subjects.

**Discussion**

No full-filled appendix was found in the 30 subjects. The partial filling appendix was 43.3% (13 subjects) and the remaining of 56.6% (17 subjects) was non-filling appendix. In the non-filling appendix (17 subjects), there were 58.8% (10 subjects) in the age group of 51-60. Followed by age group of 11-20 by 11.7% (2 subjects), 31-40 by 11.7% (2 subjects), and 21-30, 41-50, and 61-70 were consisted of 1 subject (5.8%) respectively. This phenomenon showed that not all appendices were normal full-filling following by oral contrast administration. These results were in accordance with previous study that mentioned that oral contrast was not required in appendicitis diagnosis because it was unreliable to fill the normal appendix, and also non-filling appendix was not indicated an appendix obstruction. A study by Schisgall using barium swallow in children suspected of acute appendicitis reported that 92% of partial filling and 58% of non-
visualized (non-filling) were normal appendices. A study by Sakover using barium enema in the normal appendix reported that there were 5.5% of partial filling, 12.5% of non-filling, and 21.7% appeared at the spot delay from 82% of full filling. Rice et al. mentioned that 5-10% of the normal appendix was not filled by barium. The results were in accordance with the theory that normal appendix lumen may contain contrast material, air or slightly fecal material. Thirty patients who underwent MSCT abdominal scan at the CT Unit in the Integrated Diagnostic Center Building Dr. Soetomo Teaching Hospital Surabaya were consisted of 30% male and 70% female with age range from 12 to 70 years old. Most common age group was 51-60 (50%), followed by age group of 31-40 (16.6%), 11-20 (13.3%) and the rest were evenly distributed in age group 21-30 years, 41-50 years, and 61-70 years of 6.6% respectively. This suggested that other imaging modalities with low radiation exposure will be selected at the age of the child and the reproductive age.

Schey mentioned that non-filling appendices might be due to their retro scale location. Sakover reported that the non-visualization of the appendix was related to the location of a true pelvic appendix or colitis was obtained in the right-sided colon. The absence of a full filling appendix in this study might be understandable because the normal peristaltic activity of the appendix will cause contrast in and out repeatedly. Therefore, when the picture was taken, there was no contrast filling or partially filled into the appendix lumen even though the contrast was in sufficient quantity in the cecum.

The presence of a small mucous flap covering the normal appendix orifice might functionally as a valve that allowed the appendix contents to flow out and prevented the contrast of the cecum from entering the appendix. The fact that the distal portion of the appendix might become obliterated in elderly and some might have fibrosis since birth. It should be considered as one of the causes of partial filling as well as non-filling in the normal appendix, which in this study, 50% of the sample was dominated by the age group of 51-60 (15 subjects). The ability of CT scans to visualize the appendix structure in more detail also allowed appendix filling assessment to be different compared to appendicograms. Because the appendicogram inaccurately represented the tip of the appendix, thus full filling of the appendicogram might appear as partial filling on the MSCT scan. Partial filling and non-filling might occur in normal appendices as examined using MSCT scans with oral water soluble contrast in which the ability to fill the very narrow lumen better than barium. The same conditions could occur in appendicograms that were currently being performed for appendicitis diagnostics. Meanwhile, the opinion that non-visualized/non-filling appendix with mass effect description as a pathognomonic sign for appendicitis could not be used as a reference because the mass effect might cause by the other factors such as ileitis, sesitis, small bowel obstruction, and pelvic inflammatory disease.

**Conclusion**

In MSCT abdominal scan using oral water soluble contrast, normal appendices also experienced partial filling and non-filling without any signs of obstruction, therefore the appendix lumen filling as an indicator of appendicitis was not reliable. The same results could occur in barium appendix examination (appendicogram), thus it needs to be considered when interpreting the results of appendicogram.

**Ethical Clearance:** The study has been approved by ethical committee in Dr. Soetomo Teaching Hospital Surabaya, Indonesia.

**Conflict of Interest:** The author reports no conflict of interest of this work.

**Source of Funding:** This study is done with individual funding.

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Study the Biological Efficiency of an Isolated Terpenic Compound from the Ether Petroleum Extract and the Trace Elements of the Plant Tribulusterrestris

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Department of chemistry Department of Biology, College of Science and University of Basrah College of Science and University of Basrah

Abstract

Many different compounds with a spread of biological properties and chemical structures are known from genus Tribulusterrestris together with steroid and terpinoid. In this study we tend to indicate the preliminary phytochemical analysis for rock oil ether extract and isolated compound. The results unveiled that there was no alkaloids, supermolecule , glycosides, saponins and tannic acid in petroleum ether however contain stereo and terpinoid. The isolated compound contained solely terpinoid compound. attention procedure was last this compounds. the FTIR qualitative analysis knowledge of isolated compound showed discovered absorptions signal . ultraviolet illumination spectrum showed most absorption at 220 nm due to $\pi \rightarrow \pi^*$ transition that is taken into account as characteristic feature of the unsaturated covalent bond. The concentration of the weather analyzed during this study decrease within the order Fe>Ca>Cu>Zn>Cr. important totally different among microorganism toward T. terrestris oil with p<0.05 and LSD 15.4 ,highest MIC by E.coli about 50 mm in 100% concentration of T. terrestris oil compared with control.

Keywords: Tribulusterrestris, Petroleum ether Extract, Infrared, UV-visible spectroscopy and Antibacterial

Introduction

Puncture tracheophyte genus Tribulusterrestris a spermatophyte within the rosid dicot family, native to heat temperate and tropical regions of the southern Europe and Southern Asia. This plant contain saponins ,flavonoid, sterols fatty oils ,terpenoids and metallic element salts.T. terrestris utilized in people medicines as a tonic, aphrodisiac, palliative, astringent, stomachic, antihypertensive drug, diuretic, lithotrithic, and urinary disinfectant. The edible fruit of the herb is extremely effective in most of the gu tract disorders. it’s a significant constituent of GokshuradiGuggul, a potent Ayurvedic medication wont to support correct functioning of the gu tract and to get rid of the urinary stones[1]. T. terrestris has been used for hundreds of years in writing to treat impotence, genital diseases, and sexual infirmity. In Bulgaria, the plant is employed as a people medication for treating impotence. additionally to all or any these applications, the Ayurvedic accumulation of Republic of India attributes cardiotonic properties to the foundation and fruit. In ancient Chinese drugs, the fruits were used for treatment of eye hassle, edema, abdominal distension, emission, morbid mucus, and sexual disfunction. T. terrestris represented as a extremely valuable drug within the Shern-Nong accumulation (the oldest well-known pharmacologic add China) in restoring the depressed liver, for treatment of fullness within the chest, mastitis, flatulence, acute rubor, headache, and skin problem. In Unani drugs, T. terrestris is employed as drug, delicate laxative, and general tonic[2,3]. T. terrestris could be a yearling plant cosmopolitan within the Mediterranean and also the heat elements of Europe, Asia, USA, continent and Australia [4]. This plant is employed in ancient drugs in China, India, Iraq, Iran, etc. Studies show that T. terrestris contains steroids, saponins, flavonoids, alkaloids, unsaturated fatty acids, vitamins, tannins, resins, potassium, nitrate, aminoalkanoic acid and aminoalkanoic acid [5]. This plant has several medical effects including: antimicrobial, medicinal drug, antioxidant and anti-toxic. The plant is additionally used for the treatment of vas diseases, cancers, metabolic process diseases and joint pain [6], the utilization of T. terrestris extract will increase body’s ability to create muscle mass and strength and additionally improves circulation and gas transfer [7]. future use of T. terrestris causes dilatation
of the vessels and improves coronary arteries while not facet effects \cite{8}.

Trybstan is one in every of the T. terrestris compounds that will increase concupiscence and counteract of cold-natured physiological state and biological time disorders. T. terrestris extract will increase sexual efficiency in men by increasing level of free androgenic hormone and control the number of sex hormone, progestogen and pregnenolone\cite{9}. Further, the employment of the plant in ancient drugs in Asian country and China is to treat sexual pathology and increase concupiscence by increasing the amount of androgenic hormone and gonadotropic hormone. T. terrestris had no impact on the organs like the prostate, seminal vesicles, womb and duct that are sensitive to system \cite{10}. On the opposite hand, using T. terrestris with alternative herbs as well as ginseng, soy and berries improves erectile and sexual behavior. Finally, chemical derived from T. terrestris had a big impact in reducing liquid body substance aldohexose levels that might introduce the plant as a potent healthful plant for the treatment of polygenic disorder \cite{11}.

**Materials and Method**

**Plant Material:** Puncture vine \textit{T. terrestris} classified from the local markets \textit{\textbackslash} College of Science Basrah University, the dried fruits cleaned and blended by using (Electrical Mill blender). The powder of fruits kept until required.

**chemical and Materials** : All chemicals were of purity analytical grades : hydrochloric acid (analar), n-hexane, ethyl acetate and petroleum ether from (BDH); acetone, 95% ethanol from Baghdad factory for drugs and cosmetics (Whatmann 540) filter paper.

**Petroleum ether Extract:** 20,000 gm of \textit{T. terrestris} powder was added to a thimble and then placed in a Soxhlet extractor. Heat was applied to a round bottom flask which contain petroleum ether solvent was placed at the base of the Soxhlet extractor. The extract was concentrated using a rotary evaporator (PuchiRotavapor-Re) then dried at room temperature (12), the weight of brown oil was 3.967 gm.

**Isolation methods with Column Chromatography**

Petroleum ether extract (2,000gm) was subjected to column chromatography on silica gel (73–240mesh – Merck) packed and eluted with mixture of n-Hexane: ethyl acetate(7:3).

**Thin Layer Chromatography**

(TLC) were carried out on the petroleum ether extract and isolated compound using n-Hexane: ethyl acetate(7:3)

**Preliminary Qualitative Tests**

Preliminary tests were carried out on the petroleum ether extract and isolated compound

**Infrared and UV- visible spectroscopy**

IR spectra using PyE-UNICAM-30300S Infra red spectrophotometer and Uv-visible spectra on JASCO UV \textit{T. terrestris} was weighted and digested in (5:1) mixture of nitric acid and perchloric acid (10 ml). After digestion few drops of concentrated HCl added .The solution was diluted with distilled deionized water . The dilute filtrate solution was used for analysis of minerals by atomic absorption spectrophotometer \cite{13}.

**Antibacterial to isolated compound**

It has been getting negative and positive Cram bacteria from the Department of Life Sciences / College of Science / University of Basra

**Determination of minimum inhibition concentration of \textit{T. terrestris} oil**

1ml inoculum of bacteria was added to 100 ml of nutrient broth and then incubated at 30°C for 24h and then dilution of bacterial solution with Physiological Normal saline compared with the standard test tube McFarland for 108 cells / ml of stuck bacterial and inoculated into nutrient agar , supplemented with different concentrations of the compound chemical (100%, 75%, 50%, 25%, 12.5%), using L-shap to work hole by puller diameter 6 mm, and then incubated dishes in the incubator for 37 º. Then measured the diameter of the inhibition and compared with the control \cite{14}.

**Result and Discussion**

Herbal medication was practiced in several components of the planet, thanks to its low value and affordability. Qualitative phytochemical analysis of various components of the plants and distinguishing its compounds helps practitioners in treating diseases. It conjointly helps researchers in synthesizing new chemical substances. Puncture vascular plant T. terrestris
L. could be a healthful plant wide used as a drug provider in pharmaceutical business [15]. Table (1) indicate the preliminary phytochemical analysis for fossil oil etherextract and isolated compound. The results discovered that there was no alkaloids, sugar, glycosides, saponins and phenol in petroleum ether however contain, steroid and terpinoid. The isolated compound contained solely terpinoid compound. tender loving care procedure was endure this compounds and also the results were shown in table (2) as refer[16].

Table (1): Preliminary qualitative test for petroleum ether extract and isolated compound

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Petroleum ether extract</th>
<th>Isolated compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycoside</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sterols</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table (2): TLC for preliminary qualitative test for petroleum ether extract

<table>
<thead>
<tr>
<th>Test sample</th>
<th>P-ansaldehyde&amp; Phosphoric acid</th>
<th>Ninhydrin</th>
<th>Folin reagent</th>
<th>Drangdroff</th>
<th>40% H2SO4</th>
<th>H2SO4 2ml+ Chloroform</th>
<th>visible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether extract</td>
<td>0.68</td>
<td>0.41</td>
<td>0.32</td>
<td>0.26</td>
<td>0.68</td>
<td>0.32</td>
<td>0.68</td>
</tr>
<tr>
<td>Isolated compound</td>
<td>0.68</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.68</td>
<td>0.68</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Figure(1) the FTIR spectroscopic of isolated compounds
Table (3): Infrared absorption peaks and their related function groups for isolated compounds

<table>
<thead>
<tr>
<th>Bond frequency (cm⁻¹)</th>
<th>Band</th>
<th>Mod of vibration</th>
<th>Functional groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>1173.64 cm⁻¹</td>
<td>C-H</td>
<td>Ro.</td>
<td>CH₂</td>
</tr>
<tr>
<td>1406.01 cm⁻¹</td>
<td>C-H</td>
<td>Str.</td>
<td>-CH₃ of CH₃ group</td>
</tr>
<tr>
<td>1730.03 cm⁻¹</td>
<td>C=O</td>
<td>Str.</td>
<td>Ester</td>
</tr>
</tbody>
</table>

Fig(1) and Table(3) the FTIR spectroscopic data of isolated compounds showed observed absorptions signal at 1730.03 cm⁻¹ and 1650.95 cm⁻¹ for (C=O stretching), 1406.01 cm⁻¹ (C-H in CH₃) and, 1173.64 cm⁻¹ C-H scissor for CH₂, 1053.06 cm⁻¹ C-H rocking for CH₂ and 877.55.

Fig(2) UV spectrum to isolated compound

Table (4) UV spectrum to isolated compound

<table>
<thead>
<tr>
<th>Type spectrum</th>
<th>maximum absorption</th>
<th>transition</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV spectrum</td>
<td>220 nm</td>
<td>ππ*</td>
</tr>
</tbody>
</table>

UV spectrum showed maximum absorption at 220 nm due to ππ* transition which is considered as characteristic feature of the unsaturated double bond Figure(2) and table (4). The medicinal plants serve as a good source of vital mineral elements which can be consumed both for dietary and medicinal purposes. However, the collected mineral content (Ca, Zn, Fe, Cu, Cr) of *T. terrestris* [17], Table (5) The concentration of the elements analyzed in this study decrease in the order Fe>Ca>Cu>Zn>Cr.

Table (5) shows the elements and the amount of elements found in plant *Tribulus terrestris*

<table>
<thead>
<tr>
<th>Quantity of elements measured mg/g</th>
<th>Elements</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.23</td>
<td>Calcium</td>
</tr>
<tr>
<td>3.10</td>
<td>Chrome</td>
</tr>
<tr>
<td>8.20</td>
<td>Copper</td>
</tr>
<tr>
<td>20.12</td>
<td>Iron</td>
</tr>
<tr>
<td>4.33</td>
<td>Zinc</td>
</tr>
</tbody>
</table>
Determination of minimum inhibition concentration of *Tribulus terrestris* oil

Significant different among bacterial toward *T. terrestris* oil with *p*≤0.05 and LSD 15.4 as shown. Gram negative bacteria showed high significant difference in inhibition to *T. terrestris* oil, due to differences between gram positive and gram negative bacteria include the thickness of the cell membrane, that is just about twenty to thirty nanometers thick in gram positive and eight to twelve nanometers thick in Gram negative; the number of supermolecule within the cell walls; and also the conjugated protein content, that is low in gram positive bacterium and high in gram negative. All bacterium contain a layer of peptidoglycan in their cell membrane, however the distinction between gram positive and gram negative bacterium is that Gram negative contains a skinny layer of peptidoglycan placed between 2 supermolecule layers this is often what provides Gram negative bacteria a sensitive to matter result compared gram positive bacteria[18].

From the results in table (6) highest MIC by *E. coli* about 50 mm in 100% concentration of *T. terrestris* oil compared with control. *Staphylococcus aureus* and *Bacillus cereus* were showed simple difference in MIC compared with control and showed differential and specific MIC of chemical compound impact of the growth of bacteria used to study and this showed that the impactiveness of biological or poisonous substance plant adversely have an effect on the physiology of microorganism because it contains terpenoids vehicles have substance to the expansion of bacteria effect through its impact on the method of the article organic phenomenon and so its impact on the composition of proteins contribute to the formation of necessary enzymes within the metabolism[19].

### Table (6): Minimum inhibitory concentrations (MICs) of *T. terrestris* oil on gram negative and positive bacteria (diameter of inhibition measured with millimeter)

<table>
<thead>
<tr>
<th>Concentrations of Tribulusterrestris oil %</th>
<th>Control</th>
<th>Bacillus cereus</th>
<th>Escherichia coli</th>
<th>Staphylococcus aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0</td>
<td>15</td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td>75</td>
<td>0</td>
<td>11.4</td>
<td>37.5</td>
<td>16.2</td>
</tr>
<tr>
<td>50</td>
<td>0</td>
<td>0.7</td>
<td>20.3</td>
<td>10</td>
</tr>
<tr>
<td>25</td>
<td>0</td>
<td>0</td>
<td>11.1</td>
<td>0</td>
</tr>
<tr>
<td>12.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Ethical Clearance. Authors Contribution; All authors have made substantial, direct and intellectual contribution to the work and approved it for publication.

Ethics Statement; This article contain studies with human participants performed by the authors, the approval of collecting sample from the parents of the persons of the donors.

Funding; None

Conflicts of Interest; The authors are declare there is no conflicts of interest

Data availability; All datasets generated analyzed during this study are included in the manuscript

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Medico-legal Significance of Diatoms Detection in Different Organs of Drowning Victims

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Abstract

Background: Drowning is a main universal community health problem & In Medico-Legal Aspect the autopsy diagnosis of drowning presents one of the major problems especially when there is delay in recovering the victim from water. This study concentrates on the significance of diatoms detection in different organs to reach more accurate diagnosis of drowning.

Objective: To determine the diagnostic features of drowning depending on detection of diatoms in deep tissues of drowning victims.

Methods: This study was performed at Medico-legal Directorate (M.L.D.) in Baghdad for (12) months within the period from 1/1/2018-31/12/2018. Full proper autopsy including external and internal examination of the body for all cases was performed, after obtaining complete medico-legal history, in addition to diatoms detection in different organs of drowning victims to determine the cause of the death as due to drowning.

Results: The study included (60) cases, (52) male and (8) female with their ages ranged between (15-44 years old) for male, while ages ranged between (1-44 years old) for females. Drowning was the cause of death in all cases. Among the most important results of this study is the presence of diatoms in the different organs of the drowning cases. The diatoms were found in the lung in all drowning cases (100%), and found in other organs in a percentage that increased with the duration of immersion.

Conclusion: Drowning is the 10\textsuperscript{th} cause of violent deaths in Baghdad. And important point in the diagnosis of drowning by detection of diatoms in different organs of drowning cases and pennate diatoms was common type in Iraqi waters and also study showed Aulacasiera granulata sp. common in Iraq.

Keywords: Medico-legal, diatoms detection, Drowning.

Introduction and Literature Review

Drowning is the process of experiencing respiratory impairment from submersion / immersion in liquid as stated by WHO. (Van, et al, 2005; Joost, et al, 2016) In Medico-legal view drowning is a type of asphyxia due to aspiration of fluid either water, milk, oil ...etc instead of air following immersion of whole body or nose and mouth under the level of fluid. (Hussein, 1999; Hassan, 1980; Ali, 1976; Al-Jabri, 2000). In drowning there is relation between liquid and air junction at the entrance of the airway prevents breathing air. (Idris, et al, 2003) Drowning could be considered as a mixture of mechanical presence of water within the respiratory system (mechanical asphyxia) with liquid and electrolyte changes depending on the medium (either sea or fresh water) in which immersion has occurred. (Simpson, 2011)
Autopsy Signs include those of immersion and drowning signs. Immersion signs are Maceration of the skin, becomes wrinkled, pale and wet so-called ‘washerwoman’s skin’. Cutis anserina is a common sign in immersed bodies but is connected to cold rather than warm water and cause a generalized pimpling of the skin. (Knight, 2004)

The hypostasis of bodies is frequently pink, this color is caused by the presence of unreduced oxyhemoglobin in the superficial blood vessels, but has no diagnostic value. (Joost, et al, 2016; Knight, 2004)

Mud, coal-slurry, oil, silt or sand present on the body, in addition to other artefacts such as seaweed, waterweed, algae. Mud may be adherent to the whole-body surface and clothing. Sand may be found deep in the respiratory passages and stomach, especially if the body has been rolled by the waves on a beach. But deep penetration is not sign of live aspiration. (Knight, 2004)

Other features include contraction of scrotum, and algae growth on the skin. (Knight, 2004; Roll, 2004; Nandy, 1996)

Drowning signs include froth in the air passages as a positive sign in fresh bodies. Froth is also seen around mouth and nostrils. (Knight, 2004)

Froth also observed in epilepsy, electrical shock, drug intoxication and cardiogenic pulmonary edema. (Simpson, 2011; Forens, et al, 1998)

The most important organ to observed the cause of death in drowning are lungs. They are distended brick red in color, with signs of emphysema. (Knight, 2004; Hallery, 2003)

Miscellaneous signs in drowning include bloody or watery fluid in the intracranial sinuses, engorgement of solid organs, reduced weight of the spleen, Tardieu spot on organ and muscular hemorrhages in the neck. (Simpson, 2011)

Cadaveric spasm is a positive sign and may be see in one or both hands. There may be grass, herbs, or gravel in the fist of the victim. (Simpson, 2011; Knight, 2004)

Diatoms are unicellular algae, eukaryotic microorganism measuring from 5 micron -3 mm (Bate, 2004), belong to the class of Bacillariophyceae which includes in excess of (15 000) species living in sea & fresh water. The skeleton of these algae is called a frustule which is constituted by two valves fitting together to enclose the cytoplasm (Ludes et al., 2003), and made of hard silica. Due to this hard smilacaceous skeleton, diatoms can be recovered from putrefied or injured tissues due to enzymatic or acid digestion. (Ludes, et al, 1999)

Diatoms are non-motile. (Crawford, et al, 1990) Diatoms are responsible for about 20–25% of global oxygen production. (Smolt, et al, 2010) Not all diatoms are able to penetrate the body organs through the lung cavity. The diatoms which can penetrate through this capillary network are called Drowning Associated Diatoms (DAD). (Modell, et al, 1999) A medicolegal & algological collaboration in lasting several years aimed at developing methods for dealing with cases found in water where the circumstances are not clear. (Lunetta, 2005) The specimens for diatom detection are taken from bone marrow, sphenoidal sinus aspirate, lung, liver, both kidneys, and brain. About 25gm of specimens to be taken without any contamination and collected in a clean glass bottle. (Pollanen, 1998) The acid resistant extracted material is isolated from specimens with some water from the drowning medium and would be examined microscopically to detect diatoms. (Lunetta, 2005)

Diatom test is used for diagnosis of drowning is based on the present diatoms in the medium where drowning had taken place. The inhaled water enters the alveolar spaces of lungs and penetrates from the alveoli into the blood circulation, thus diatoms will be transported to different organs of body. The diatoms found inside the organs of drowned victim is a conclusive evidence to support the diagnosis of death by drowning. Diatoms are classified as Class Bacillariophyceae that is divided into two Orders. The Centric (Biddulphiales) which have valve striae arranged basically in relation to a point, an annulus or a central areola and tend to appear radially symmetrical as in figure (1). The Pennates (Bacillariales), which have valve striae arranged in relation to a line and tend to appear bilaterally symmetrical as in figure (2), their silica-based skeletons do not readily decay, and they can sometimes be detected even in heavily decomposed bodies. (Kaushik, 2017)
This study is aimed to determine the diagnostic significance of diatoms detection in different internal organs of victims in drowning cases.

**Subjects and Methods:**

**Site and Duration:** Medico-legal Directorate (M.L.D.) of Baghdad for one year duration from 1/1/2018 till 31/12/2018.

**Subjects:** sixty victims of drowning. Inclusion criteria: Cases with circumstantial evidence favoring drowning death. Exclusion criteria: Decomposition.

**Method**

Full forensic autopsy consisting of external examination including that of the clothes and external signs followed by internal examination after taking history of the incident from the autopsy request form written by the police authority and from the relatives of the victims. Tissue samples were taken from different organs for detection of diatoms type and species, and this includes:

1. Taking specimens (about 1 gram) from lungs, heart, liver, kidneys and brain.
2. Putting them in a mixture of ethanol alcohol (20%) and (80%) distilled water.
3. Taking bone pieces from sternum or femurs and washing them in distilled water. Longitudinal sections to be made using a clean band saw, then taking sample of (1 g) to be placed into a mixture of ethanol alcohol (20%) and (80%) distilled water.
4. Keeping samples in a cool atmosphere (refrigerator).
5. Sending samples to the toxicology lab. to investigate them for diatoms.

**Laboratory investigation for Diatoms:**

One gram from each organs are taken and placed in glass bake. Sulphuric acid diluted with distilled water (30%, 70%) is added until the volume is twice that of the original sample. The sample then will be placed at room temperature over night to dissolve the organic matter. Sulphuric acid removes resistant dirts In this method no foaming will be produced. After that centrifugation will be done in test tube with (4000) rpm for (10) min. The supernatant will be discarded and sediment washed several times by distilled water. The previous process are repeated (3 – 4) times. From each sample (100) microliter is taken, spread on a surface of slide and dried in oven at (70 - 90°C). The slides are examined under light microscope (Novel type). The diatoms were identified according to morphology & species. Diatoms resist putrefaction, so diatom test is valuable even if the decomposition is advanced. Diatom test is negative in dead bodies thrown in water and in dry drowning. In diatom examination, the control water samples must be used for comparison purpose. Standard diatom samples can be preserved on slides and can be used as standards for comparison purpose. The test is positive if minimum of (20) diatoms are identified in each (100 µl) of sediment extracted from lung sample and if more than (5) diatoms in each (100 µl) of sediment extracted from a tissue sample.
Results

The study included (60) cases, (52) male and (8) female with their ages ranged between (15-44 years old) for males, while ages ranged between (1-44 years old) for females. Drowning was the cause of death in all cases.

This study showed the presence of diatoms in different organs of the drowning victims. The diatoms were found in the lung in (100% = 60) of cases, in the liver in (41.7% = 25) of cases, in the kidney in (40% = 15) of cases, and there were no diatoms in bone marrow and brain (if the duration of immersion is about 30 minutes). Other results were obtained in other durations of immersion as shown in figure (3).

![Figure 3: Presence of diatoms in samples of different organs according to the duration of immersion in water.](image)

This study showed that there are two types of diatom in fresh water, the first is pennate diatom in a percent of about (75%) or in (45) cases. The second type is centric diatoms in a percent of (25%) or in (15) cases as in figure (4).

![Figure 4: Diatoms type in drowning cases.](image)
The Aulacasiera granulata sp. was the most common in fresh water in this study (in about 43.3% of cases =26). While Amoeba sp. was discovered in about (3.3%) or (2) cases as in Table (1). See also figure (5) & (6).

**Table 1: Diatoms species in drowning cases.**

<table>
<thead>
<tr>
<th>Diatoms species in drowning cases</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aulacasiera granulata sp.</td>
<td>26</td>
<td>43</td>
</tr>
<tr>
<td>Cyclotella sp.</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>Nitzschia filiforms sp.</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td>Gurosigma sp.</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td>Amoeba sp.</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>100</td>
</tr>
</tbody>
</table>

**Figure 5:** - Pennate diatom, shows the filamentous diatom *Aulacosiera granulata* x 400

**Figure 6:** - Centric diatom, *Cyclotella* sp. x 400
Discussion

In this study the diatoms were found in the lungs in all drowning’s victims if the period of immersion is about 30 minutes as in Figure(3) because the lungs are the first invaded by water in drowning. While in other organs, such as the liver, kidneys, brain and bone marrow the presence and percentage of diatoms varies according to the duration of immersion in water. Those results agrees with an Indian study. (Kaushik, 2017)

The study also showed the presence of diatoms in the body organs of the drowning victims in all cases due to the fact that the person was alive during drowning and he inhaled water containing diatoms that moved to various organs of the body, so diatoms detection in samples taken from different organs of victims especially those other than lung and stomach are very helpful in diagnosis of drowning as it is a challenge in forensic practice. (Robert; Stemberg, 2009)

The Pennate type of diatoms was commoner than the centric in this study. The commonest species respectively were Aulacasiera granulata sp., Cyclotella sp., Nitzschia filiforms sp., Gurosigma sp., Amoeba sp., were found to be frequent respectively in Iraqi water Table(1) in agreement with the above mentioned Indian study. (Kaushik, 2017)

The detection of diatoms in the lungs alone (Found in all cases in this study) is also helpful in the diagnosis of drowning. The result that was also found in a study from srilanka. (Gunatilake, 2010)

Conclusion

Diatoms detection in samples from lung and different other internal organs of drowning victims are very helpful in the diagnosis of drowning cases in addition to the traditional external and internal findings. Diatoms were present in the lung in all cases of drowning, while diatoms detection in other organs was in relation with the period of immersion of the victim.

Diatoms of Pennate type specially that of Aulacasiera granulata sp. was the most common in Iraq.

References

15. Ludes, B., Fornes, P., Drowning in, Forensic Medicine, Clinical and Pathological Aspects,


Evaluation of Nurses’ Knowledge and Attitudes toward Pain Management at Baghdad Teaching Hospitals

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Abstract

Poorly managed or unmanaged pain is a problem that affects individuals, entire healthcare systems and societies throughout the world.

Objectives: To assess nurses’ knowledge and attitudes about patient’s pain management and to find out the relationship between knowledge, attitudes and their demographical characteristic.

Methodology: A descriptive cross sectional design study was carried out among 100 nurses working in male and female surgical wards, ICU, surgical ward, emergency and operating theatre of four teaching hospitals, Baghdad city. Census method was used to select the participants, in which all the nurses working in the selected wards who agreed to participate in the study were selected. Data was collected by distributing structured self-administered questionnaire which was a modified form of a standard tool, that is, Nurses’ Knowledge and Attitude Survey Regarding Pain (NKASRP) developed by Ferrel and McCaffery.

Results: The majority of the study were male who accounted for (63%) of the total participants while female constituted (37%). Most of participants (49%) were ages between (18 – 27) years old, a high percentage of them were have institute graduate (40%), (50%) of the nurses were single and the remainder was married. Majority of them (37%) were employee (1-5) years in employment in nursing. Majority of them (45%) were employee (1-5) years in intensive care unit, and finally majority of nurses (77%) have training session about patient’s pain management.

Conclusions: This study indicated that nurses’ have poor knowledge about pain management, as well as study indicated overall nurses’ have positive attitudes toward pain management.

Keywords: Evaluation, Nurses’, Knowledge, Attitudes, Pain Management

Introduction

Pain is defined as unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage (¹). Chronic and acute pain differs in both mechanism and duration. Acute pain is a behavioral or biochemical cascade initiated by tissue injury (²). Pain is the major symptom that brings patients to the health care setting and is also the commonest symptom that hospitalized patients encounter in general and in surgical settings in particular. Pain management practices are the activities that are provided by nurses and other health care professionals to ensure that the pain is managed effectively (³). An examination of the prevalence of acute pain in hospitalized adult patients, by way of a systematic review, revealed a range of 37.7%-84.0% of patients reporting acute pain (⁴). Nurses are key contributors to inter professional pain management teams, and are involved in both the assessment and management of pain. It is important to understand pain knowledge and attitudes amongst nursing. Effective pain management is a vital component to quality patient care. Nurses play an essential role in implementing pain management.

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Methodology

A descriptive cross sectional study design was applied for assessing knowledge and attitude of nurses on pain management. The study site was Baghdad teaching hospital, Kase AL- Harere teaching hospital, Al-Kindey teaching hospital and Al- Karama teaching hospital in Baghdad city. The selected wards for the study were male and female surgical wards, ICU, surgical ward, emergency and operating theatre. These wards were selected purposively as the nurses working in these areas have to deal more often with pain management. All the nurses working in the selected wards who agreed to participate in the study were selected. The total number of nurses working in the selected wards was 150. However, as few nurses refused to participate in the study, the total sample size was 100. Modified after literature review and discussion with the experts as well as considering the national context. The tool’s test-retest reliability was \( r = 0.85 \) and the internal consistency reliability was alpha \( r = 0.75 \). Each correct answer was scored 1 and there was no negative marking for wrong answer. The data was collected through distribution of self-administered questionnaire. The instrument comprised of pre-designed structured questionnaire which included demographic information, 22 multiple choice questions and 10 questions related to nurses’ attitude. The standard tool was used, that is, Nurses’ Knowledge and Attitude Survey Regarding Pain (NKASRP) by Ferrel and McCaffery (5). The data collected from 1st October 2018 to 15th July 2019. The data were analysed through statistical analysis was done using statistical package for social Sciences (SPSS v23.0).

Results

The majority of the participants were male (63%) of the total participants while female constituted (37%). (49%) of participants were ages between (18 – 27) years, majority of them were have institute graduate (40%), (50%) of the nurses were single and the remainder was married. (37%) were employee (1-5) years in nursing, (45%) were employee (1-5) years in ICU, and finally (77%) of nurses’ have training session about pain management.

<table>
<thead>
<tr>
<th>No</th>
<th>Items</th>
<th>correct</th>
<th>ncorrect</th>
<th>MS</th>
<th>A.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Most accurate judge of the intensity of pain is (patient’s)</td>
<td>83.0</td>
<td>.17</td>
<td>.83</td>
<td>G</td>
</tr>
<tr>
<td>2</td>
<td>Pain should be assessed before &amp; after administering drugs.</td>
<td>84.0</td>
<td>16.0</td>
<td>.84</td>
<td>G</td>
</tr>
<tr>
<td>3</td>
<td>Side effects of narcotics should be observed at least 30 minute after administration</td>
<td>42.0</td>
<td>58.0</td>
<td>.42</td>
<td>P</td>
</tr>
<tr>
<td>4</td>
<td>A pain rating scale, is appropriate for patients to use to rate their pain.</td>
<td>79.0</td>
<td>21.0</td>
<td>.79</td>
<td>G</td>
</tr>
<tr>
<td>5</td>
<td>A patient’s pain should be assessed at rest and during mobilization</td>
<td>40.0</td>
<td>60.0</td>
<td>.40</td>
<td>P</td>
</tr>
<tr>
<td>6</td>
<td>Pain assessment is based on the patient’s behavior and physiological changes.</td>
<td>82.0</td>
<td>18.0</td>
<td>.82</td>
<td>G</td>
</tr>
<tr>
<td>7</td>
<td>Patients with a history of substance abuse should not be given.</td>
<td>56.0</td>
<td>44.0</td>
<td>.56</td>
<td>F</td>
</tr>
<tr>
<td>8</td>
<td>The most common side effect of morphine.</td>
<td>25.0</td>
<td>75.0</td>
<td>.25</td>
<td>P</td>
</tr>
<tr>
<td>9</td>
<td>The recommended route of administration of opioid for pts with persistent cancer-related pain.</td>
<td>22.0</td>
<td>78.0</td>
<td>.22</td>
<td>P</td>
</tr>
<tr>
<td>10</td>
<td>Analgesic is considered drug of choice for the treatment of prolonged moderate to severe pain for cancer patients</td>
<td>44.0</td>
<td>56.0</td>
<td>.44</td>
<td>P</td>
</tr>
<tr>
<td>11</td>
<td>Analgesics for postoperative pain should initially be given. Around the clock on a fixed schedule</td>
<td>25.0</td>
<td>75.0</td>
<td>.25</td>
<td>P</td>
</tr>
<tr>
<td>12</td>
<td>The most likely reason a pt with pain would request increased doses of pain medication.</td>
<td>54.0</td>
<td>46.0</td>
<td>.54</td>
<td>F</td>
</tr>
<tr>
<td>13</td>
<td>How likely it is those pts who develop pain already have an alcohol &amp;/ drug abuse15min</td>
<td>53.0</td>
<td>47.0</td>
<td>.53</td>
<td>F</td>
</tr>
</tbody>
</table>
Cont. Table (1) : Mean of Score of Nurses’ knowledge domain concerning Patient Pain Management.

<table>
<thead>
<tr>
<th>No</th>
<th>Items</th>
<th>Strong agree</th>
<th>Agree</th>
<th>Disagree</th>
<th>MS</th>
<th>AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>The time to peak effect for morphine given orally</td>
<td>53.0</td>
<td>47.0</td>
<td>.47 P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Patients may sleep in spite severe pain</td>
<td>18.0</td>
<td>82.0</td>
<td>.18 P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Valium is not effective pain relievers unless the pain is</td>
<td>42.0</td>
<td>58.0</td>
<td>.42 P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Vital signs indicators of the intensity of a patient’s pain</td>
<td>8.0</td>
<td>92.0</td>
<td>.08 P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Respiratory depression rarely occurs in pt take opioid</td>
<td>60.0</td>
<td>40.0</td>
<td>.60 F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>useful for treatment of cancer pain</td>
<td>28.0</td>
<td>72.0</td>
<td>.28 P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>statement is true regarding opioid induced respiratory depression</td>
<td>41.0</td>
<td>59.0</td>
<td>.41 P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>A 30 mg dose of oral morphine is approximately equivalent to morphine</td>
<td>41.0</td>
<td>59.0</td>
<td>.41 P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>The most likely reason a patient with pain would request increased doses of pain medication</td>
<td>80.0</td>
<td>20.0</td>
<td>.80 G</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>48.1</td>
<td>51.9</td>
<td>.42 p</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(A.D.): Assessment Degree, M.s=mean of score [(0 - .49) = Poor knowledge (F); (0.5 – .74) = Fair knowledge (F); (0.75 – 1) = Good Knowledge(G)]

Table (2) Mean of Score of Nurses’ Attitudes domain concerning Patient’s Pain Management

<table>
<thead>
<tr>
<th>No</th>
<th>Items</th>
<th>Strong agree</th>
<th>Agree</th>
<th>Disagree</th>
<th>MS</th>
<th>AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pain is seen in the patient’s behavior</td>
<td>58.0</td>
<td>39.0</td>
<td>3.0 2.55 G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Distraction reduces pain intensity</td>
<td>43.0</td>
<td>50.0</td>
<td>7.0 2.36 G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Non-pharmacological interventions are very effective for mild to moderate pain</td>
<td>39.0</td>
<td>44.0</td>
<td>17.0 2.22 A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>The use of placebo is important in determining if the patient’s pain is real</td>
<td>57.0</td>
<td>41.0</td>
<td>2.0 2.55 G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Medical patients usually do not experience pain which is as intense as that experienced by surgical patient</td>
<td>58.0</td>
<td>37.0</td>
<td>5.0 2.53 G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>When a medical patient complains of pain the best management is to assess the genuineness of the pain</td>
<td>44.0</td>
<td>51.0</td>
<td>5.0 2.39 G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Using pain assessment tools usually makes nursing more complicated and consumes time for other ward activities</td>
<td>28.0</td>
<td>61.0</td>
<td>11.0 2.17 A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Medical patients who complain of pain often, will be seeking staff attention</td>
<td>22.0</td>
<td>66.0</td>
<td>12.0 2.10 A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Nurses are best judges of the patient’s pain intensity because they spend 24 hours with the patient</td>
<td>51.0</td>
<td>37.0</td>
<td>12.0 2.39 G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Because patients are not medically educated cannot give a reliable report of their pain</td>
<td>61.0</td>
<td>31.0</td>
<td>8.0 2.53 G</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(A.D.): Assessment Degree, M.s=mean of score [(1 – 1.66) = poor (p); (1.67 – 2.33)= Acceptance(A) ; (2.34 – 3) = Good (G)]
Table 3: Association between Nurses Knowledge and socio demographic variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>df*</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>2</td>
<td>.685</td>
<td>1.145</td>
<td>.323</td>
</tr>
<tr>
<td>Level of Education</td>
<td>2</td>
<td>.432</td>
<td>.538</td>
<td>.585</td>
</tr>
<tr>
<td>Experience Years in Hospital</td>
<td>2</td>
<td>1.766</td>
<td>3.039</td>
<td>.052</td>
</tr>
<tr>
<td>Experience Years in ICU</td>
<td>2</td>
<td>2.261</td>
<td>3.281</td>
<td>.042</td>
</tr>
<tr>
<td>Training course</td>
<td>2</td>
<td>.251</td>
<td>1.417</td>
<td>.247</td>
</tr>
</tbody>
</table>

*: P ≤ 0.05; **: P ≤ 0.01, χ², t-test

Table 4: Association between Nurses Attitudes and various factors

<table>
<thead>
<tr>
<th>Variables</th>
<th>df*</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>2</td>
<td>.885</td>
<td>1.490</td>
<td>.230</td>
</tr>
<tr>
<td>Level of Education</td>
<td>2</td>
<td>2.000</td>
<td>2.595</td>
<td>.080</td>
</tr>
<tr>
<td>Experience Years in Hospital</td>
<td>2</td>
<td>4.229</td>
<td>2.364</td>
<td>.099</td>
</tr>
<tr>
<td>Experience Years in ICU</td>
<td>2</td>
<td>.282</td>
<td>.387</td>
<td>.680</td>
</tr>
<tr>
<td>Training course</td>
<td>2</td>
<td>.420</td>
<td>2.416</td>
<td>.095</td>
</tr>
</tbody>
</table>

Discussion

Throughout the course of the data analysis of the current study, the findings show the majority of the study were male who accounted for (63%) of the total participants while male constituted (37%). Most of the study participants (49%) were ages between 18 and 27 years old. A high percentage of them were institute graduate (40%), most of them (50%) were single, (37%) were for (1-5) years were employment in nursing, Majority of them (45%) were employee (1-5) years in surgical wards, and finally majority of nurses (77%) have training session about patient pain management.

These findings agreed with study done by Benimama, who reported that majority of participants 34 (39.5%) are aged between 31-35 years old; (22.1%) are ranged between (26-30) years and (18.6%) are aged between (36-40) years, majority of participants 37 (43%) have between (3-10) years of experience in nursing (5,6,7).

Fifty three questions assessed general knowledge of patient pain management. Overall (48.1%) of the participants answered knowledge questions correctly and (51.9%) responded “Incorrect answer”. All general question knowledge concerning method used to reduce or relieve patient pain through non-pharm logical and pharm logical intervention for patients suffering pain. The table (1) informs us that participants have scored 80% and above in only (4) questions. They scored between 50% and 79% in (5) questions and they scored less than 50% in thirteen questions. The highest score was 84%. The table also shows that (83%) knew that most accurate judge of the intensity of the patient’s pain, (84%) of nurses’ answers that pain should be assessed before and after administering drugs. Only (42%) knew...
that side effects of narcotics should be observed at least 30 minutes after administration. Ninety seven of them asked pain rating scale, is appropriate for patients to use to rate their he/she pain, low percent of participation answer A patient’s pain should be assessed at rest and during mobilization. It also show that (82%) of total population study reported that pain assessment is based on the patient’s behaviour and physiological changes. It also shows that (56%) knew the meaning of opioid addiction, (25%) knew that obstructive sleep apnea is the risk factor of opioid induced respiratory depression, only (25%) knew the analgesics for postoperative pain should initially be given around the clock on a fixed schedule. It shows that (54%) knew that then patient will request increased doses of pain medications because he/she is experiencing increased pain. (54%) knew that likely it is those pts. who develop pain already have an alcohol / drug abuse15 minute, (47%) knew the time to peak effect for morphine given orally is15 minute and (18%) knew the patients may sleep in spite although severe pain. These findings agreed with study done by Benimana who reported that the (79.1%) knew that respiratory depression can occur in patients receiving opioids, and (89.5%) knew that sedation assessment must be done during opioid pain management because of excessive sedation that precedes respiratory depression. Only 27(31.4%) knew that obstructive sleep apnea is the risk factor of opioid induced respiratory depression. It shows that (70.9%) knew that after the initial dose of opioid is given, other doses will be adjusted according to patient’s response. It also shows that 71(82.6%) knew the meaning of opioid addiction, and only (22.1%) knew the manifestations of physical dependence when there is abrupt discontinuation of an opioid (5) These findings are similar to Yosef as he found that pharmacological knowledge of anaesthetists regarding post-operative pain management in Ethiopia were poor yet being knowledgeable about pharmacology of opioid analgesics was prerequisite for all health care professionals in the effective pain management (8). Kizza, reported that most of participants had knowledge deficits and bad attitudinal beliefs which lead to ineffectual pain management among surgical patients (9). Another study done by Craig that found poor knowledge and bad attitudes of nurses towards pain management (10). The results are also supported by Shahriary et. al who found that oncology nurses lacked sufficient knowledge regarding pain management among cancer pain (11). The study was also supported by study done in Rwanda by Ufashingabire et al., who found that nurses working in ICU had poor knowledge and bad attitudes towards pain assessment and management (12).

This finding was in good agreement with that obtained by Tufekci et al. have identified knowledge deficits about pain management and health care professionals misconceptions about pain as the major challenge towards effective pain management. Among the challenges about pain management that are related to health care professionals, there is insufficient knowledge about pain assessment and management, therefore health care professionals need to have recognition of it in order to improve quality of care through pain management (13,14). The association between socio-demographic and nurses knowledge and attitudes score was explored. There are association between years of employment in nursing and studied sample knowledge at p value 0.05 and also illustrate that no relationship found with rest of studied variables. These findings are in line with study done by Irene, who reported that found no statistically significant evidence for a real difference between mean knowledge scores with respect to unit experience [6]. These findings are in line with study done by Indrah, the student t-test was employed to compare the associations between pain assessment practices and nurses demographic data namely years of experience as a registered nurse, years of experience as ICU and the qualifications (15).

Conclusions

This study demonstrate that, despite poor nurses’ knowledge regarding pain management, as well as study indicated overall nurses’ have positive attitudes toward pain management. We recommend to There is need to design and implement a continuous professional education program on pain and its assessment with special focus on methods of assessment, guidelines, how to use assessment tools, protocols and charts for proper documentation for critically ill.

Acknowledgement: This research was funded by Authors. Moreover, we would like to thank the study participants and data collectors for their fully participation and responsible data collection.

Conflict of Interest: None declared.

Ethical approval: The study was approved by the Institutional Ethics Committee.
References


15. Indrah GM, Nurses Knowledge related to Pain Assessment for Critically Patients at A public Sector Hospital in Johannesburg, dissertation, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg,2014; 152-1.
The Effect of Empowerment Program on Participation of Mothers with Premature Infants Hospitalized in Neonatal Intensive Care Unit

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Abstract

Introduction: The birth of a premature infant who needs to be admitted to neonatal intensive care unit from birth impedes early communication and participation of mother in her infant’s care. The purpose of this study was to determine the effect of empowerment program on the participation of mothers with premature infants admitted to neonatal intensive care unit.

Method: This study was a randomized clinical trial in 2018, which was conduct on a sample of mothers with premature infant who had randomly been divided into two intervention and control groups. The data collection tools were the Parents’ Participation Inventory designed by Melnick in 1994. Data were analyzed by descriptive statistics (tables, mean and standard deviation) and inferential statistics using SPSS-16 software.

Results: There was no significant difference in the level of mothers’ participation in the care of their infants between the two groups before the intervention (p = 0.45). But after the empowerment training program, the results showed that mothers’ participation in the intervention group (19.10 ± 3.09) was higher than the control group (16.8 ± 2.01), (p = 0.01).

Conclusion: The results of this study showed that implementation of empowerment training program increases mothers’ participation in the care of their premature infants. Early intervention and the use of written information along with the booklet will increase the mothers’ participation in the care of their infant.

Key words: Empowerment, Participation, Mothers of premature infants

Introduction

When the patient’s age is small events and various diseases threaten him(1, 2). Patient hospitalization poses challenges for the patient and the patient’s family(3). These events can cause more harm to the person. Infants who are born before 37 weeks from the last menstruation day are called premature infants that often weight less than 2500 grams(4). Premature or gestational age of less than 37 weeks is one of the most important health indicators in any society, and infant survival is directly related to gestational age and birth weight(5). Advances in technology and neonatal care have ensured the survival of pre-term infants and reduced their mortality rate(6, 7).

Admission of a newborn infant to a neonatal intensive care unit (NICU) is a frightening and stressful experience for parents (8, 9). Number of adverse outcomes including increased risk of mental problems such as anxiety, depression, traumatic stress disorder, poor parent-infant interactions, and emotional, behavioral and cognitive problems are associated with the premature birth. It has been well documented that, the presence of scary medical equipment in the neonatal intensive care

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Birth of a premature infant is not only an emotional and stressful experience for parents, but also an evolutionary stage of life for infants. Understanding the process of becoming a parent is difficult due to the fast and unexpected events that unfold, and having a premature infant can be a sad experience and difficult to cope with and sometime, this emotion is so severe that makes it difficult to control the situation. Numerous studies have shown that parental roles and responsibilities will change in these situations. Mothers of premature infants are less likely to have parental roles and often have doubts about their abilities to identify and meet their infant’s needs. Parents need to be able to cope with these conditions and their new role, but this process is not always easy. Undoubtedly, the parents of premature infants require support and currently, one of the best and most effective ways to prevent injuries and harm to premature infants is to actively involve their parents in their care.

The purpose of this study was to determine the effect of empowerment program on participation of mothers with premature infants hospitalized in neonatal intensive care unit of Sayyed Shirazi hospital in Gorgan, Iran.

Materials and Method

This study is a randomized clinical trial which was conducted on the two groups of mothers with preterm infants. The setting of this study was Sayyed Shirazi Hospital at the city of Gorgan, Iran. The sample size in this study was determined based on the study of Gavami et al (2012) using G*POWER software with the effect size of 0.94, significant level 0.05, confidence interval of 0.95 and test power 0.080. The samples were randomly divided into two groups of intervention (n=20) and control (n=20). Inclusion criteria were: being over 18 years old, having the ability to read and write, having no history of neonatal intensive care unit care, having no physical or mental illness leading to drug use, having a 26 to 37 weeks old infant who weigh less than 2500 grams, and lack of life-threatening condition in the infant. The simple random sampling method was used in this study. Data collection tools were demographic information questionnaire (maternal age, education, occupation, number of children) and Parents’ Participation Inventory, which was designed by Melnick in 1997 to measure parental involvement in the neonatal intensive care unit. This questionnaire lists 25 activities that a mother can perform for her infant. Also, the mother is asked to tick every activity that she has performed. The questions in this questionnaire have two options, and the higher number of options selected by the mother indicate the greater care provided by the mother and the more involvement she has in the care of her infant. A score of less than 9 indicates low level of participation, 10-18 indicates moderate level of participation, and score of 19 or more indicates the high level of participation. In Melnick’s (1997) study, the Cronbach’s alpha of this tool was 0.85.

After explaining the purpose of study, the necessary permission was obtained from the hospital authorities. According to the inclusion criteria, purposeful sampling was done in the first step and then, the study samples were randomly divided into two intervention and control groups. The researcher, after introducing herself, explained the aims of study to the mothers, ensured them about the principles of confidentiality and anonymity, asked them to provide a written informed consent and informed them that they could withdraw from the study at any time with any reason. The first appointments were made with the participating parents and the meeting place was agreed upon. The interval between each intervention step was 4 days. Three phases of the parent empowerment program were implemented for the parents in the intervention group. The control group received no intervention other than routine care and support in the ward. At the beginning and the end of the study, pre-test and post-test questions were completed by the participants. Data were analyzed by SPSS-16 software.

After the study, audio tapes containing written information and booklets used for parents in the intervention group during the study were given to the control group, so that they could also benefit from the program. The intervention was based on the following protocol:

**Session 1:** Demographic information questionnaire was competed by mothers 4 days after the infant hospitalization and beginning of the intervention in this stage. Then, a 15-minute long audio tape containing information on the first stage along with the booklet was given to the mothers. The information on this stage was
related to the appearance and behavioral characteristics of the premature infant, the differences between a pre-term and term infant, environmental characteristics of NICU, and strategies to enhance the maximum participation of parents in the infant care.

**Session 2:** It took place 4 days after the first stage. A 15-minute long audio tape containing information on the second stage along with the booklet was given to the mothers. In addition to providing support for the first stage, information on the behavior and evolutionary growth of infant and some suggestion for maximizing the parents’ participation in the care was provided.

**Session 3:** It took place one day before the infant discharge. In addition to providing support for the last two stages, information on discharge, how to care for infant at home, how to identify the characteristics of behavior and evolutionary growth of infant and signs of distress were given to parents. After the intervention, mothers completed the post-test.

**Findings**

The results of independent t-test showed no significant difference between the two groups in terms of mothers’ age (p = 0.62) and length of hospital stay (p = 0.27). In the intervention group, 0.09 of the samples (N=18) were housewives and 0.010 (N = 2) were employed. Also, in the control group, 0.080 of the samples (N = 41) were housewives and 0.020 (N = 4) were employed. The result of chi-square test showed no significant difference between the two groups in terms of mother’s occupation (p = 0.66), history of childbirth (p = 0.25), delivery method (p=0.35), history of abortion (p = 0.26), and type of pregnancy (p = 0.36). The Fisher test showed no significant difference between the two groups in terms of education (p = 0.69), and the Mann-Whitney test also showed no significant difference between the two groups in terms of number of children (p = 0.31).

Chi-square test did not show a significant difference between the two groups before and after the intervention (p = 0.45). Also, the independent t-test showed no significant difference between the two groups in terms of the mean score of level of participation (p=0.21), (Table 1).

Chi-square test showed a significant difference between the two groups in terms of the level of participation after the intervention (p=0.01), so that the level of participation increased to 55% (N=11). Paired t-test showed a significant difference between the two groups in terms of the level of participation before and after the intervention (p=0.008), (Table 1).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intervention</th>
<th>Control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level of participation</td>
<td>frequency</td>
<td>Percentage</td>
<td>frequency</td>
</tr>
<tr>
<td>Moderate</td>
<td>9</td>
<td>45</td>
<td>16</td>
</tr>
<tr>
<td>High</td>
<td>11</td>
<td>55</td>
<td>4</td>
</tr>
<tr>
<td>Mean &amp; SD</td>
<td>19.10±3.09</td>
<td>16.8±2.01</td>
<td></td>
</tr>
</tbody>
</table>

Paired t-test showed a significant difference in the level of participation in the intervention group before and after the intervention (p<0.01, t=11.2). It also showed a significant difference in the control group (p<0.01, t=-10.82), but the level of participation was more in the intervention group (Table 3).

**Table 2: Comparison of the level of parents’ participation in the intervention and control groups before and after the intervention.**

<table>
<thead>
<tr>
<th>Time</th>
<th>Intervention group</th>
<th>Control group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before intervention</td>
<td>9.2 ± 3.63</td>
<td>10.35 ± 1.89</td>
<td>P=0.21</td>
</tr>
<tr>
<td>After intervention</td>
<td>19.1 ± 3.09</td>
<td>16.8 ± 2.10</td>
<td>P=0.008</td>
</tr>
<tr>
<td>p-value</td>
<td>p&lt;0.01</td>
<td>p&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>t-value</td>
<td>t=11.2</td>
<td>t=10.82</td>
<td></td>
</tr>
</tbody>
</table>
Covariance test showed a significant difference before the intervention (p=0.004, Eta=0.2), so that 20% of the changes in parents’ level of participation was due to the empowerment training (Table 3).

**Table 3: The effect of empowerment training program on the level of parents’ participation**

<table>
<thead>
<tr>
<th>Variance source</th>
<th>Sum of squares</th>
<th>Degree of freedom</th>
<th>Mean of squares</th>
<th>F-value</th>
<th>Significant level</th>
<th>Eta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modified model</td>
<td>70.49</td>
<td>2</td>
<td>35.24</td>
<td>5.4</td>
<td>P=0.009</td>
<td>0.22</td>
</tr>
<tr>
<td>Post-test separator</td>
<td>17.59</td>
<td>1</td>
<td>17.59</td>
<td>2.69</td>
<td>P=0.1</td>
<td>0.06</td>
</tr>
<tr>
<td>Group</td>
<td>63.41</td>
<td>1</td>
<td>63.41</td>
<td>9.71</td>
<td>P=0.004</td>
<td>0.2</td>
</tr>
<tr>
<td>Error</td>
<td>241.4</td>
<td>37.5.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sum</td>
<td>13200</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>311.90</td>
<td>39</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Conclusion**

Nursing interventions improve patients’ health\(^{18, 19}\). Findings of the present study showed that mothers with premature infants who had an empowerment training program were more involved in the care of their infants. In a study by Melnick et al., four stages of the “creating opportunities for parent empowerment” (COPE) were implemented in the United States\(^{6}\). In this program, parents learned about the premature infant, family’s abilities and activities that parents can perform. Also, psychological support was provided for parents and sufficient time was given to them for preparation. Consequently, the confidence of parents in caring for their premature infants increased\(^{20}\). Jaw Brown conducted a study on parents of premature infants that showed that the knowledge of mothers increased after the training. Providing information on infant behavior and interaction with infant reduce mothers’ stress and have positive effects on the level of parents’ participation in the care of their infants\(^{16}\).

Results of studies by Fatemeh Alaei Karahroudi et al. (2012) showed that COPE program had a positive effect on mothers’ participation in the care of their infant and increased it in the intervention group\(^{20}\). Therefore, empowerment model can be considered as an appropriate model for promoting health, increasing knowledge and enhancing parents’ participation in the care of their infants\(^{21}\). Study of Abdolali Zadeh et al. (2015) showed that a health promotion support program is effective in promoting the quality of life in mothers of premature infants\(^{22}\). Empowering and involving parents and families in the care and decision-making for their infants promote health and wellbeing. People who are able to control their emotions can make the right decision\(^{23}\).

**Conclusion**

Considering the results and the impact of empowerment program on the participation of parents in the care of their premature infants, it can be said that the implementation of empowerment program can facilitate active participation of parents in the care of their premature infants. Thus, we suggest to implement this program from the first day of delivery for mothers of premature infants.

**Conflict of Interest:** There is no conflict of interest between authors.

**Acknowledgement:** We would like to express our gratitude to the officials of Golestan University of Medical Sciences and the neonatal intensive care unit of Sayyed Shirazi Hospital in Gorgan.

**Source of Funding:** Islamic Azad University of Aliabad Katoul.

**Ethical Clearance:** IR.GOMUS.REC1397.092 approved by Golestan University of Medical Sciences
References


Association of Diffusion Weighted Magnetic Resonance Imaging Profile and Apparent Diffusion Coefficient Value with Brain Tumor’s Histopathology

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Abstract

Background: Accuracy of preoperative brain tumor’s histopathologic differentiation is very important to determine preoperative staging, intraoperative management and postoperative therapy. Diffusion weighted magnetic resonance imaging is an advanced technique developed as an important method for brain tumor assessment.

Objectives: This study aimed to investigate correlation between diffusion weighted imaging (DWI) profile and apparent diffusion coefficient (ADC) value with brain tumor’s histopathology.

Methods: This study examined 86 subjects with brain tumors using magnetic resonance imaging and histopathology examination at Dr. Soetomo Teaching Hospital and evaluated DWI profile and ADC maps. The spherical ROI on ADC maps was placed on the solid part of the tumor to measure ADC value. Spearman’s correlation test was conducted to determine correlation between DWI profile and ADC values with histopathological grading and types of brain tumor.

Results: There was a significant correlation between DWI profile and ADC values (p = 0.000), as well as DWI and ADC values with histopathological grading and types of brain tumor (p = 0.000). There was an inverse correlation between ADC value and histopathological grading. The higher the ADC value, the lower the brain tumor grade, while lower ADC value resulted in high tumor grade. The average ADC value of grade-1 meningiomas was 1.061±0.257 x 10⁻³ mm²/sec, pilocytic astrocytoma was 1.301±0.107 x 10⁻³ mm²/sec, glioblastoma multiforme was 0.831±0.080 x 10⁻³ mm²/sec, and medulloblastomas was 0.600±0.078 x 10⁻³ mm²/sec.

Conclusion: Evaluation on DWI profile and ADC value could provide additional information on conventional magnetic resonance imaging examination to determine histopathological grading and types of both intra and extra-axial brain tumors.

Keywords: ADC value, brain tumor, brain tumor histopathology, DWI.

Introduction

Accurate brain tumor diagnosis has an important role in selecting an optimal therapeutic strategy due to the influence of tumor natural course and grading on therapeutic approach of preoperative, intraoperative and postoperative management. Conventional CT scan and MRI have limitations in differentiating intracranial lesions, with sensitivity, specificity, PPV and NPV of conventional MRI to determine high grade glioma. Therefore, many advanced MRI techniques are developed to improve the accuracy of preoperative brain tumor histopathologic diagnosis.

Diffusion weighted imaging (DWI) is an advanced MRI technique that is currently being developed as an
important method for assessing brain tumor. In general, malignant tumors have large cell nuclei and exhibit high cell density. A study has shown that malignant tumors have low ADC values than benign tumors. Therefore, one of the most prominent contributions of DWI is the differentiation between malignant and benign brain tumors. The sensitivity, specificity, PPV and NPV and ADC values of DWI in differentiation of high-grade brain tumors and lymphomas with low-grade glioma and non-neoplasm cases were 94.1%, 78.2%, 76.1% and 94.7%, respectively.

Recently, there has been no data on DWI profile and ADC of brain tumor which is associated with histopathology examination result, especially in Dr. Soetomo General Hospital, Surabaya, Indonesia. These data are important to show whether there is a difference with previous research. They also can be used as a reference in determining the diagnosis and characterization of preoperative brain tumor using MRI examination. Therefore, we aimed to examined correlation of DWI profiles and ADC values with histopathological outcomes in both postoperative patients with intra-axial and extra-axial brain tumors.

**Method**

A retrospective study investigated correlation of DWI profile and ADC value with histopathology grading and type of brain tumor in post-brain tumor surgery patients at Dr. Soetomo Teaching Hospital, Surabaya, Indonesia. The subjects were patients with brain tumor who visited Neurosurgery Unit for head examination, surgery and histopathology examination.

All subjects should meet the following inclusion criteria: (1) Patients with intra-axial and extra-axial brain tumors; (2) Having a complete and accessible medical record; (3) Having performed head MRI with contrast and DWI sequence; (4) Having performed surgery and histopathological examination of tumor tissue. We excluded subjects with the following criteria: (1) There was no MRI file or have not performed any DWI sequence; (2) Intratumoral bleeding; (3) Intratumoral inflammatory process.

Subjects were taken using consecutive sampling technique. We used GE Optima 360 1.5 Tesla MRI machine. MRI examination protocol used head coil, while sequences made included T1WI using fast spin echo with Time repetition (TR) 250-750 ms and Time echo (TE) 10-20 ms (SE 250-750/10-20), T1WI + C, T2WI sequence (SE 4000-8000/100). DWI with b value of 1000 mm²/sec was performed on axial pieces. ADC is a reconstruction of the DWI sequence. ADC value measurement in ROI tumors was round with size of 20-30 mm². It was placed in the denser area of the tumor that experienced signal intensity changes on DWI. ADC value measurement in normal brain parenchyma ROI had round shape of 20-30 mm², and it was placed in white matter on the counter-lateral side of the lesion. Gadolinium-DPTA (Omniscan, GE Healthcare Inc.) media contrast was administered intravenously to all patients with a dose of 0.1 mmol/kg WW.

Data processing and analysis begin with an evaluation of MRI examination results conducted by two neuroradiologists (ADS) and (WFA) who did not know of tumor tissue’s histopathological examination results. Decisions were made by means of agreement if there were any difference in MRI interpretation.

Descriptive analysis was conducted to obtain the sample’s characteristics. We conducted Kappa test to determine interobserver variation on the interpretation of MRI head examination. Spearman’s correlation test was then performed to determine the correlation between DWI profile and ADC values with grading and histopathology type in brain tumor. The confidence limit used was 95% (95% CI) with significant p value <0.05. The statistical calculations used SPSS software (SPSS. Inc. Chicago. IL).

**Results**

We obtained 97 samples aged 1-82 years old, with average age of 33.39 years. Most subjects were found in the age group of 41-50 years (n = 24, 24.74%) followed by age group of 31-40 years (n = 23, 23.71%) and age group of less than 10 years (n = 21, 21.65 %). Table 1 showed subjects’s distribution by Tumor Histopathology. Table 1 showed subjects’ distribution by tumor histopathology.
Table 1. Subjects’ Distribution by Tumor Histopathology

<table>
<thead>
<tr>
<th>Histopathology</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meningioma transitional</td>
<td>19</td>
<td>19.59</td>
</tr>
<tr>
<td>Pituitary adenoma</td>
<td>14</td>
<td>14.43</td>
</tr>
<tr>
<td>Medulloblastoma</td>
<td>9</td>
<td>9.28</td>
</tr>
<tr>
<td>GBM</td>
<td>6</td>
<td>6.19</td>
</tr>
<tr>
<td>Meningioma meningoethelial</td>
<td>6</td>
<td>6.19</td>
</tr>
<tr>
<td>Pilocytic astrocytoma</td>
<td>6</td>
<td>6.19</td>
</tr>
<tr>
<td>Schwannoma</td>
<td>6</td>
<td>6.19</td>
</tr>
<tr>
<td>Meningioma atypical</td>
<td>4</td>
<td>4.12</td>
</tr>
<tr>
<td>Meningioma microcyst</td>
<td>4</td>
<td>4.12</td>
</tr>
<tr>
<td>Diffuse fibrillary astrocytoma</td>
<td>3</td>
<td>3.09</td>
</tr>
<tr>
<td>Anaplastic ependymoma</td>
<td>2</td>
<td>2.06</td>
</tr>
<tr>
<td>Meningioma fibroblastic</td>
<td>2</td>
<td>2.06</td>
</tr>
<tr>
<td>CNS PNET</td>
<td>2</td>
<td>2.06</td>
</tr>
<tr>
<td>Meningioma microcyst and angiomatous</td>
<td>2</td>
<td>2.06</td>
</tr>
<tr>
<td>Adamantinoma craniopharyngioma</td>
<td>1</td>
<td>1.03</td>
</tr>
<tr>
<td>Anaplastic astrocytoma</td>
<td>1</td>
<td>1.03</td>
</tr>
<tr>
<td>Anaplastic oligodendroglioma</td>
<td>1</td>
<td>1.03</td>
</tr>
<tr>
<td>Atheroma (dermoid cyst)</td>
<td>1</td>
<td>1.03</td>
</tr>
<tr>
<td>Choroid plexus carcinoma</td>
<td>1</td>
<td>1.03</td>
</tr>
<tr>
<td>Choroid plexus papilloma</td>
<td>1</td>
<td>1.03</td>
</tr>
<tr>
<td>Ependymoma clear cell</td>
<td>1</td>
<td>1.03</td>
</tr>
<tr>
<td>Germinoma</td>
<td>1</td>
<td>1.03</td>
</tr>
<tr>
<td>Mature teratoma</td>
<td>1</td>
<td>1.03</td>
</tr>
<tr>
<td>Meningioma microcyst and transitional</td>
<td>1</td>
<td>1.03</td>
</tr>
<tr>
<td>Psammomatous meningioma</td>
<td>1</td>
<td>1.03</td>
</tr>
<tr>
<td>Pilomyxoid astrocytoma</td>
<td>1</td>
<td>1.03</td>
</tr>
<tr>
<td>Total</td>
<td>97</td>
<td>100</td>
</tr>
</tbody>
</table>
ADC value characteristic of normal brain parenchyma by age showed that the age <17 years old have mean 0.865 \((10^{-3} \text{ mm}^2/\text{sec})\) with SD 0.045 and the age >17 years old have mean 0.820 \((10^{-3} \text{ mm}^2/\text{sec})\) with SD 0.057. ADC value of WHO grade I tumor was 1.152±0.042 \((10^{-3} \text{ mm}^2/\text{sec})\), WHO grade II was 1.063±0.081 \((10^{-3} \text{ mm}^2/\text{sec})\), WHO grade III was 0.865±0.080 \((10^{-3} \text{ mm}^2/\text{sec})\), WHO grade IV was 0.603±0.065 \((10^{-3} \text{ mm}^2/\text{sec})\). ADC value of facilitated and restricted diffusions in brain tumors were 1.139±0.037 \((10^{-3} \text{ mm}^2/\text{sec})\) and 0.644±0.057 \((10^{-3} \text{ mm}^2/\text{sec})\), respectively. The average ADC value of facilitated and restricted diffusions in extra-axial brain tumor were 1.138±0.042 \((10^{-3} \text{ mm}^2/\text{sec})\) and 0.642±0.083 \((10^{-3} \text{ mm}^2/\text{sec})\), respectively. The average ADC value of facilitated and restricted diffusions in intra-axial brain tumor were 1.140±0.075 \((10^{-3} \text{ mm}^2/\text{sec})\) and 0.646±0.074 \((10^{-3} \text{ mm}^2/\text{sec})\), respectively.

**Table 2. Brain tumor characteristics by DWI profiles and ADC value**

<table>
<thead>
<tr>
<th>Brain Location</th>
<th>Histopathology</th>
<th>WHO Grading</th>
<th>DWI Profile</th>
<th>ADC Value ((\times 10^{-3} \text{ mm}^2/\text{sec}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-axial</td>
<td>Pilocytic astrocytoma</td>
<td>I</td>
<td>Facilitated</td>
<td>1.408 0.285</td>
</tr>
<tr>
<td>Diffuse fibrillary astrocytoma</td>
<td>II</td>
<td>Facilitated</td>
<td>1.295 0.361</td>
<td></td>
</tr>
<tr>
<td>Anaplastic astrocytoma</td>
<td>III</td>
<td>Facilitated</td>
<td>1.440</td>
<td></td>
</tr>
<tr>
<td>Anaplastic oligodendroglioma</td>
<td>III</td>
<td>Facilitated</td>
<td>0.970</td>
<td></td>
</tr>
<tr>
<td>Anaplastic ependymoma</td>
<td>III Restricted</td>
<td>0.728</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNS PNET</td>
<td>IV</td>
<td>Restricted</td>
<td>0.430</td>
<td></td>
</tr>
<tr>
<td>Medulloblastoma</td>
<td>IV Restricted</td>
<td>0.596 0.864</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GBM</td>
<td>IV</td>
<td>Facilitated</td>
<td>0.831 0.209</td>
<td></td>
</tr>
<tr>
<td>Extra-axial</td>
<td>Psammomatous meningioma</td>
<td>I</td>
<td>Facilitated</td>
<td>0.812</td>
</tr>
<tr>
<td>Meningioma microcystic and angiomatous</td>
<td>I</td>
<td>Facilitated</td>
<td>0.940</td>
<td></td>
</tr>
<tr>
<td>Meningioma microcystic and transitional</td>
<td>I</td>
<td>Facilitated</td>
<td>0.866 0.113</td>
<td></td>
</tr>
<tr>
<td>Meningioma meningothelial</td>
<td>I</td>
<td>Facilitated</td>
<td>0.914</td>
<td></td>
</tr>
<tr>
<td>Meningioma transitional</td>
<td>I</td>
<td>Facilitated</td>
<td>0.979 0.129</td>
<td></td>
</tr>
<tr>
<td>Meningioma fibroblastic</td>
<td>I</td>
<td>Facilitated</td>
<td>1.079 0.142</td>
<td></td>
</tr>
<tr>
<td>Pituitary adenoma</td>
<td>I</td>
<td>Facilitated</td>
<td>1.045 0.304</td>
<td></td>
</tr>
<tr>
<td>Schwannoma</td>
<td>I</td>
<td>Facilitated</td>
<td>1.047 0.106</td>
<td></td>
</tr>
<tr>
<td>Adamantinoma craniopharyngioma</td>
<td>I</td>
<td>Facilitated</td>
<td>1.660</td>
<td></td>
</tr>
<tr>
<td>Choroid plexus papilloma</td>
<td>I</td>
<td>Facilitated</td>
<td>2.092</td>
<td></td>
</tr>
<tr>
<td>Pilocytic astrocytoma</td>
<td>I</td>
<td>Facilitated</td>
<td>1.194</td>
<td></td>
</tr>
<tr>
<td>Meningioma atypical</td>
<td>II</td>
<td>Facilitated</td>
<td>0.962 0.084</td>
<td></td>
</tr>
<tr>
<td>Pilomyxoid astrocytoma</td>
<td>II</td>
<td>Facilitated</td>
<td>1.408</td>
<td></td>
</tr>
<tr>
<td>Anaplastic ependymoma</td>
<td>III Restricted</td>
<td>0.710</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meningioma anaplastic</td>
<td>III Restricted</td>
<td>0.658</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choroid plexus carcinoma</td>
<td>III Restricted</td>
<td>0.685</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNS PNET</td>
<td>IV Restricted</td>
<td>0.551</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medulloblastoma</td>
<td>IV Restricted</td>
<td>0.605 0.033</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3 showed a significant correlation between DWI profile and ADC values \((p = 0.000)\). The DWI profile showed a significant correlation with histopathology grading \((p = 0.000)\), and ADC value significantly correlated with histopathology grading \((p = 0.000)\).

Table 3. Correlation of DWI Profile and ADC Value with Brain Tumor’s Histopathology Grading

<table>
<thead>
<tr>
<th>Location</th>
<th>WHO Grading</th>
<th>DWI Profile</th>
<th>ADC Value ((x 10^{-3}\text{mm}^2/\text{sec}))</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-axial</td>
<td>I</td>
<td>Facilitated</td>
<td>1.130</td>
<td>0.450</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Restricted</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>Facilitated</td>
<td>1.185</td>
<td>0.109</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Restricted</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>Facilitated</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Restricted</td>
<td>0.684</td>
<td>0.113</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>Facilitated</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Restricted</td>
<td>0.578</td>
<td>0.120</td>
<td></td>
</tr>
<tr>
<td>Extra-axial</td>
<td>I</td>
<td>Facilitated</td>
<td>1.408</td>
<td>0.087</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Restricted</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>Facilitated</td>
<td>0.940</td>
<td>0.120</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Restricted</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>Facilitated</td>
<td>1.205</td>
<td>0.138</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Restricted</td>
<td>0.728</td>
<td>0.195</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>Facilitated</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Restricted</td>
<td>0.619</td>
<td>0.470</td>
<td></td>
</tr>
</tbody>
</table>

Spearman’s correlation between ADC value and brain tumor location \((r = 0.119; p = 0.227)\)

Spearman’s correlation between ADC value and DWI profile \((r = 0.563; p = 0.000)\)

Spearman’s correlation between WHO grading and DWI profile \((r = 0.930; p = 0.000)\)

Spearman’s correlation between ADC value and WHO grading \((r = 0.514; p = 0.000)\)

Table 4 showed a significant correlation between ADC value and histopathology of brain tumors \((p = 0.000)\).
Table 4. Correlation of ADC Value and Brain Tumor’s Histopathology

<table>
<thead>
<tr>
<th>WHO Grading</th>
<th>Histopathology</th>
<th>ADC Value (x 10^-3 mm²/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>I</td>
<td>Pilocytic astrocytoma</td>
<td>1.301</td>
</tr>
<tr>
<td></td>
<td>Choroid plexus papilloma</td>
<td>2.092</td>
</tr>
<tr>
<td></td>
<td>Adamantinoma craniopharyngioma</td>
<td>1.660</td>
</tr>
<tr>
<td></td>
<td>Schwannoma</td>
<td>1.047</td>
</tr>
<tr>
<td></td>
<td>Pituitary adenoma</td>
<td>1.045</td>
</tr>
<tr>
<td></td>
<td>Meningioma fibroblastic</td>
<td>1.080</td>
</tr>
<tr>
<td></td>
<td>Meningioma meningothelial</td>
<td>0.914</td>
</tr>
<tr>
<td></td>
<td>Meningioma microcyst and transitional</td>
<td>0.938</td>
</tr>
<tr>
<td></td>
<td>Meningioma microcyst and angiomatous</td>
<td>0.866</td>
</tr>
<tr>
<td></td>
<td>Meningioma microcyst</td>
<td>0.940</td>
</tr>
<tr>
<td></td>
<td>Psammomatous meningioma</td>
<td>0.812</td>
</tr>
<tr>
<td>II</td>
<td>Ependymoma clear cell</td>
<td>0.585</td>
</tr>
<tr>
<td></td>
<td>Diffuse fibrillary astrocytoma</td>
<td>1.295</td>
</tr>
<tr>
<td></td>
<td>Pilomyxoid astrocytoma</td>
<td>1.408</td>
</tr>
<tr>
<td></td>
<td>Atypical meningioma</td>
<td>0.962</td>
</tr>
<tr>
<td>III</td>
<td>Anaplastic meningioma</td>
<td>0.658</td>
</tr>
<tr>
<td></td>
<td>Anaplastic astrocytoma</td>
<td>1.440</td>
</tr>
<tr>
<td></td>
<td>Anaplastic oligodendroglioma</td>
<td>0.970</td>
</tr>
<tr>
<td></td>
<td>Anaplastic ependymoma</td>
<td>0.719</td>
</tr>
<tr>
<td></td>
<td>Choroid plexus carcinoma</td>
<td>0.685</td>
</tr>
<tr>
<td>IV</td>
<td>Medulloblastoma</td>
<td>0.600</td>
</tr>
<tr>
<td></td>
<td>GMB</td>
<td>0.831</td>
</tr>
<tr>
<td></td>
<td>CNS PNET</td>
<td>0.491</td>
</tr>
</tbody>
</table>

Spearman’s correlation between ADC value and brain tumor’s histopathology (r = .563; p = .000)

**Discussion**

In this study, the average value of normal brain parenchyma’s ADC white matter in age group less than 17 years was 0.865±0.045 x 10^-3 mm²/sec, while 0.820±0.057 x 10^-3 mm²/sec for age group more than 17 years. This value was higher than the normal value found in a study conducted by Thomas study (0.75±0.03 x 10^-3 mm²/sec)⁴.

We found a significant correlation between DWI profile and ADC value (p = 0.000). The average ADC value in DWI profile of facilitated and restricted diffusions in brain tumor were 1.139±0.037 x 10^-3 mm²/sec and 0.644±0.057 x 10^-3 mm²/sec, respectively. In another study, ADC value less than 1.0-1.1 x 10^-3 mm²/sec indicated a restricted diffusion of water molecules⁵. Nevertheless, we found no significant correlation between ADC value and brain tumor location. The value of facilitated and restricted diffusions in intra-
axial and extra-axial brain tumors showed no significant difference.

MRI is a highly sensitive imaging modality for evaluating brain tumors, but conventional MRI has limitations to differentiate histopathologic types of most brain tumors. In this study, we found a significant relationship between ADC value and tumor histopathology grading (p = 0.000). This finding was consistent with a study that found higher ADC value led to LGG (WHO I, II), while lower ADC value led to HGG (WHO III, IV). These findings were consistent with a study that found low ADC value led to atypical or malignant meningiomas compared with benign 6.

Increased mitotic processes, necrosis, high cytoplasmic nucleus ratios and increased disturbed cell growth patterns are found in high-grade meningiomas, causing restricted diffusion of water molecules seen in DWI. On the other hand, benign meningiomas show little representation of a coherent histological organization, because they are composed of oval-shaped neoplastic or spindle cells that form threads, fascicles, cords, or nodules, which force water molecules to move relatively isotropic 1. The location of pituitary adenoma could be affected by the air-induced susceptible artifacts in the sinuses and surrounding bones resulting in varying results on the evaluation of ADC values, but no data supporting this possibility has been obtained 7. On the other hand, PCL as a hyperseleruler tumor has been reported to have lower ADC values significantly greater than HGG and metastatic tumors1. A study reported that lymphoma and metastasis’ ADC values were lower than glioma8.

A study (n = 76) reported ADC tumor metastasis was $0.72 \times 10^{-3} \text{mm}^2/\text{sec}$ (using b-value of 1,000 mm$^2$/sec) 9. On the other hand, another study (n = 21) reported ADC tumor metastasis values ranging from 0.35 to 1.37 x $10^{-3} \text{mm}^2/\text{sec}$, with an average of $0.79\pm0.23 \times 10^{-3} \text{mm}^2/\text{sec}$ (using b-value of 1,000 mm$^2$/sec). Nevertheless, the study found no significant correlation between ADC tumor metastasis score and GBM 10. However, there is limited analysis of ADC sample values with histopathology ependymoma and heterogeneous morphology and tumors, therefore the results of this group are not fully reliable and further research is needed 12.

Brain tumor is an abnormal growth of cells in the brain. Magnetic resonance imaging (MRI) is an advanced diagnostic tools that enable us to visualize anatomical details more clearly so superior in detecting abnormalities in the soft tissues of the brain 13. Neoplasia refers to the growth of new cells that are different from the growth of cells around it 14. Image segmentation has been popularly performed for researchers in the field of Biomedical, Informatics Engineering, and Statistical Computation 15.

**Conclusion**

ADC value measurement in both extra-axial and intra-axial brain tumors was a significant predictor of grading differentiation and histopathologic types of brain tumor. Clinicians could use this diagnostic method for comprehensive action planning and management of brain tumor cases.

**Ethical Clearance:** The study protocol was approved by the ethics committees of Dr. Soetomo Teaching Hospital, Surabaya, Indonesia.

**Conflict of Interest :** No conflict of interest reported from this research

**Source of Funding:** This research funded individually.

**References**

5. Haaga JR, Dogra VS, Forsting M. CT and MRI Of


Mitochondrial 16S rRNA gene-dependent Blood typing as a Forensic Tool

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Abstract

Background: Mitochondrial DNA is an important tool for human identification and is used to differentiate between human and animal blood at the crime scene, because in extreme conditions nuclear DNA is severely destroyed while Mitochondrial DNA contains multiple copies (200-2000) in per cell as well as resists harsh and more stable conditions. Methodology: Seventy-two blood samples were collected from human (Homo sapiens), sheep (Ovis aries), goat (Capra hircus) and cow (Bos taurus) (Eighteen blood samples for each). All blood samples were withdrawn by technician and 5ml were aspirated using aseptic technique and transferred to EDTA-Na2 tube and mixed well and stored in refrigerator. The collection takes 2 weeks (15th May 2019 to 30th May 2019). All samples were collected from Al-Diwanyia city. Results: The results of PCR reveal that, the primer pairs were specific and non-specific products not appear for all samples. The amplification of Homo sapiens mitochondrial DNA with primer pairs of other (Ovis aries, Capra hircus and Bos taurus) and amplification of each with primers pair of another genus gave negative results and this a primary evidence for primer pairs specificity. The amplicon of 16S rRNA gene of Homo sapiens were 1200bp, Ovis aries were 1060bp, Capra hircus were 820bp, and Bos taurus were 1300bp. The sequencing revealed that no cross-reactivity of designed primer pairs and the PCR assay based on the designed primer pairs will be simple, fast, sensitive, specific, and cost-effective. Conclusion: Sensitivity, specificity and accuracy of the designed species specific primer pairs and applicability of the designed primer pairs in forensics to investigate blood sports or evidence belonging for human, sheep, goat and cow.

Keywords: Homo sapiens, Ovis aries, Capra hircus, Bos taurus, Forensic.

Introduction

Mitochondria have their possess small spherical genome, mtDNA, which encodes for the thirteen important subunits of the electron transport chain and ATP synthase together with 22 tRNAs and 2 rRNAs necessary for mitochondrial protein synthesis[1,2]. Mitochondrial DNA presents several characteristics valuable used for forensic studies, especially attendant to the absence of recombination, to a great copy number, and to matrilineal inheritance. Mitochondrial DNA typing founded on sequences of the control region otherwise filled genomic sequence is used to examine a variation of forensic mtDNA profiling methods used for human proof of identity and present their use in the chief cases of human identification from non-human [3-5]. Mitochondrial markers that are used for species identification are as follows: cytb gene, cytochrome c oxidase subunit I gene, 12S and 16S rRNA segment and control region in wildlife[6-8]. A short fragment of the 12S rDNA was employed for DNA amplification leading to species identification. The mitochondria DNA 16S rRNA gene is an advanced genetic marker for animal genetic diversity. Utilizing gene mitochondrial DNA 16S rRNA. Polymorphism sites, nucleotide variation, and haplotype variety were determined using whole sequences of the mitochondrion DNA 16S rDNA gene[9,10]. Animal mitochondrial DNA (mtDNA) is commonly described as a small, circular molecule that

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is conserved in size, gene content, and organization \cite{11}. The aim of this study is to design valuable species specific-PCR tool to discriminate blood of human and non human Species specific- primer design.

**Methodology**

**Study Design**

The study design was experimental to design species specific primer pairs for typing the blood samples and their assignment to human (\textit{Homo sapiens}), sheep (\textit{Ovis aries}), goat (\textit{Capra hircus}) and cow (\textit{Bos taurus}).

**Blood Sample Collection:**

Seventy two blood samples were collected from human, sheep, goat and cow (Eighteen blood samples for each). All blood samples were withdrawn by technician and 5ml were aspirated using aseptic technique and transferred to EDTA-Na2 tube and mixed well and stored in refrigerator. The collection take 2 weeks (15th May 2019 to 30th May 2019). All samples were collected from Al-Diwanya city.

**Primer Design**

The gene selected for this study is mitochondrial 16S rRNA gene. the NCBI data base were used to recover the sequences chosen for primer design. The sequence ID of \textit{Homo sapiens}:(NC_012920.1); sequence ID of \textit{Ovis aries}:(NC_001941.1); sequence ID of \textit{Capra hircus} (NC_005044.2); sequence ID of \textit{Bos taurus} (NC_006853.1). Primer 3 software \cite{12} was used to design the specific primer using the sequence of above mentioned sequences ID. The generated primer were: Homo 16S-F: GCCTGGTGATAGCTGGTTGT, Homo 16S-R: ATCATTTACGGGGGAAGGCG (1200bp); Ovis 16S-F: AGGCCTAAAAGCACGCGCATCA, Ovis 16S-R: GCCCTTTCTAGGGCAGGT (1060bp); Capra 16S-F: GCCTGGTGATAGCTGGTTGT, Capra 16S-R: TCACCCCAACCAAACGCTGCT (820bp) and Bos 16S-F: CTAAGCAGCGAACCAGA, Bos 16S-R: GGGCAAGGGTGGTGGCT (1300bp).

**Mitochondrial DNA extraction**

G-spinTM Total DNA Extraction Kit(50 Preps) (REF: 17045 ) was used to extract mitochondrial DNA from blood of different species according to the manufacturer’s protocol instructions.

**Agarose gel electrophoresis**

Agarose gel was prepared by dissolving agarose powder in 1X TBE buffer. The amount of agarose which can be dissolved depending upon the purpose in which agarose sheet used. 0.7% agarose gel used for visualization the DNA after extraction while 1.5%-2% agarose sheet visualization of PCR product (amplicon). RedSafe (alternative for ethidium bromide) stock solution concentration was 10 mg/ml. Only 5μl of RedSafe stock solution were added to 100ml of melted agarose gel to get final concentration 0.5μg/ml \cite{13,14}.

**Primer pairs preparation and PCR conditions**

The primers were synthesized at (Macrogen/ Korea), were provided in a lyophilized from, which were re-dissolved with 300 nuclease-free water according to institution of manufacture company to reach to the final concentration (100 pmoles/μl). The working solution will be 10 pmoles/μl to be used directly in PCR\cite{15,16}. The PCR conditions were calculated using online Protocol Optimize writer software. The conditions were illustrated in table (1).

**Table (1): PCR conditions**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Conditions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homo 16S-F</td>
<td>95°C</td>
<td>2min.</td>
</tr>
<tr>
<td>Homo 16S-R</td>
<td>95°C</td>
<td>30sec.</td>
</tr>
<tr>
<td>30</td>
<td>59.3°C</td>
<td>30sec.</td>
</tr>
<tr>
<td></td>
<td>72°C</td>
<td>130sec.</td>
</tr>
<tr>
<td>1</td>
<td>72°C</td>
<td>5min.</td>
</tr>
</tbody>
</table>

This study
Result and Discussion

The four sets of designed primer pairs were submitted to specificity using Primer-Blast and the results revealed that, they are specific to amplify 16S rRNA gene of Human (*Homo sapiens*), Sheep (*Ovis aries*), Goat (*Capra hircus*) and Cows (*Bos taurus*) (table 2). 16S rDNA region is highly conserved region among mtDNA[17]. mtDNA can be easier to retrieve from low-quantity and/or degraded DNA samples, as it is present at many copies per cell, thus providing a clear advantage over nuclear genome-based methods of species identification[18-20].

### Table (2): Primer-Blast of designed primer pairs

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequence 5’ to 3’</th>
<th>Sequence ID of isolate</th>
<th>Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homo sapiens 16S rRNA</td>
<td>F:GCCTGGTGATAGCTGGTTGT R:ATCATTTACGGGGGAAGCG</td>
<td>MN115376.1 MN053904.1 MN125706.1 MN163828.1 MN163832.1 MN125705.1 MN163282.1 MN125704.1 MN124446.1 MK069579.1</td>
<td>100%</td>
</tr>
</tbody>
</table>
The results of PCR reveal that, the primer pairs were specific and non-specific products not appear for all samples. The amplification of Homo sapiens mtDNA with primer pairs of other (Ovis aries, Capra hircus and Bos taurus) and amplification of each with primers pair of another genus gave negative results and this a primary evidence for primer pairs specificity. The amplicon of 16S rRNA gene of Homo sapiens were 1200bp (Figure 1A), Ovis aries were 1060bp(Figure 1B), Capra hircus were 820bp (Figure 1C), and Bos taurus were 1300bp (Figure 1D). PCR amplification and sequence analysis of mitochondrial 16S rRNA gene for their use in differentiation/identification and subsequently evaluating their application in solving the forensic cases [21]. Mitochondrial 16S is suitable for the differentiation of 300 mammalian species. 16S rDNA gene is common mitochondrial gene for detection of blend mutton and pork at high sensitivity. The mitochondrial 16S rRNA genes have been used as molecular markers to identify mammals, birds, shrimp, and other species using species-specific primers that amplify the 12S rRNA or 16S rRNA gene regions from mtDNA[17,22]. Gene loci on the mitochondrial genome have been used in species identification. These include the 12S and 16S rRNA loci. The D-loop (displacement loop) has been used less in species identification but more in intraspecies identification. Due to the greater sequence variation at this non-coding locus, it is now being used as a tool for identifying the presence of particular species within mixture of many species [23,24].

Table (2): Primer-Blast of designed primer pairs

<table>
<thead>
<tr>
<th>Species</th>
<th>Primer Sequence</th>
<th>Accession Numbers</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovis aries 16S rRNA</td>
<td>F:AGGCCTAAAAGCAGCCATCA R:GCCCTTTTCTAGGGCAGGTT</td>
<td>KP998473.1, KP998472.1, KP998470.1, KP702285.1, MH841968.1, MH841967.1, MH841966.1, MG837554.1, MG837553.1, KU681224.1</td>
<td>100%</td>
</tr>
<tr>
<td>Capra hircus 16S rRNA</td>
<td>F:GCCTGGTGATAGCTGGTTGT R:TCACCCCAACCAAAACTGCT</td>
<td>LS992662.1, LS992661.1, LS992659.1, LS992658.1, LS992656.1, LS992655.1, LS992654.1, LS992653.1, LS992652.1, LS992651.1</td>
<td>100%</td>
</tr>
<tr>
<td>Bos taurus 16S rRNA</td>
<td>F:CTAAGCAGCCCGAAACCAGA R:GGGCAGGGTTTTGTGTTGC</td>
<td>EU177866.1, EU177865.1, EU177864.1, EU177863.1, EU177862.1, EU177861.1, EU177860.1, EU177859.1, EU177858.1, EU177856.1</td>
<td>100%</td>
</tr>
</tbody>
</table>
The secondary and confirmatory assay for specificity of primer pairs used in study is sequences of PCR products. Eight amplicons from each were sent for sequencing using Sanger technique (Macrogen/Korea). The retrieved sequences firstly must be trimmed to remove unwanted sequences before submitting them for BLASTN. The trimming performed by Bioedit to get the finally processed sequences. Abbreviation of *homo sapiens* sequences were used as (HIS-1 to HIS-8), *Ovis aries* sequences be (IOA-1 to IOA-8), *Capra hircus* sequences be (IBCH-1 to IBCH-8) and *Bos taurus* sequences be (IBT-1 to IBT-8).

The identity percentage and alignment results of amplified 16S rRNA gene of *homo sapiens*, *Ovis aries*, *Capra hircus* and *Bos taurus* with database were illustrated in table (3,4,5,6) respectively.

Figure 1: Agarose gel electrophoresis 1.5% for: A: 1200bp amplicon of *Homo sapiens* 16S rRNA gene. lane H1-H18 represent samples. B: 1060bp amplicon of *Ovis aries* 16S rRNA gene. lane H1-H18 represent samples. C: 820bp amplicon of *Capra hircus* 16S rRNA gene. lane H1-H18
Table (3): Identity of blasted isolates (IHS-1 to IHS-8) with reference sequences of highest identity percentage

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Sequence ID</th>
<th>Expect</th>
<th>Identities</th>
<th>Gaps</th>
<th>Strand</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHS-1</td>
<td>MH444415.1</td>
<td>0.0</td>
<td>98.98%</td>
<td>0/885(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IHS-2</td>
<td>MK069579.1</td>
<td>0.0</td>
<td>99.74%</td>
<td>0/771(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IHS-3</td>
<td>MK069579.1</td>
<td>0.0</td>
<td>99.57%</td>
<td>2/697(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IHS-4</td>
<td>MK069579.1</td>
<td>0.0</td>
<td>99.08%</td>
<td>0/654(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IHS-5</td>
<td>MK059695.1</td>
<td>0.0</td>
<td>99.86%</td>
<td>0/701(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IHS-6</td>
<td>MK069579.1</td>
<td>0.0</td>
<td>99.39%</td>
<td>0/657(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IHS-7</td>
<td>MK295855.1</td>
<td>0.0</td>
<td>99.50%</td>
<td>0/599(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IHS-8</td>
<td>MK069579.1</td>
<td>0.0</td>
<td>99.69%</td>
<td>0/653(0%)</td>
<td>Plus/Plus</td>
</tr>
</tbody>
</table>

Table (4): Identity of blasted isolates (IOA-1 to IOA-8) with reference sequences of highest identity percentage

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Sequence ID</th>
<th>Expect</th>
<th>Identities</th>
<th>Gaps</th>
<th>Strand</th>
</tr>
</thead>
<tbody>
<tr>
<td>IOA-1</td>
<td>MG489885.1</td>
<td>0.0</td>
<td>98.70%</td>
<td>0/769(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IOA-2</td>
<td>MG489885.1</td>
<td>4e-170</td>
<td>100.00%</td>
<td>0/329(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IOA-3</td>
<td>MG489885.1</td>
<td>0.0</td>
<td>98.98%</td>
<td>0/586(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IOA-4</td>
<td>MG489885.1</td>
<td>0.0</td>
<td>99.73%</td>
<td>0/749(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IOA-5</td>
<td>MG489885.1</td>
<td>0.0</td>
<td>99.63%</td>
<td>0/542(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IOA-6</td>
<td>MG489885.1</td>
<td>2e-157</td>
<td>99.36%</td>
<td>0/312(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IOA-7</td>
<td>MG489885.1</td>
<td>0.0</td>
<td>99.17%</td>
<td>2/483(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IOA-8</td>
<td>MG489885.1</td>
<td>0.0</td>
<td>99.80%</td>
<td>0/489(0%)</td>
<td>Plus/Plus</td>
</tr>
</tbody>
</table>
The sequencing of the 16S rRNA has revolutionized the study and identification of human and non-human in forensic. Many study development a simple method using universal primers for species identification based on direct PCR sequencing using primer sets were designed based on the conserved regions of the 16S rRNA loci detected by the comprehensive sequence comparison among 30 animals whole[25]. Mitochondrial DNA the method could be a dominant tool for mammalian species identification, especially in forensic cases in which many unidentified biological samples must be analyzed such as blood spots[25]. The 16S and 12S sequences

**Table (5): Identity of blasted isolates (IBCH-1 to IBCH-8) with reference sequences of highest identity percentage**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Sequence ID</th>
<th>Expect</th>
<th>Identities</th>
<th>Gaps</th>
<th>Strand</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBCH-1</td>
<td>KP271023.1</td>
<td>0.0</td>
<td>99.02%</td>
<td>0/614(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IBCH-2</td>
<td>KP271023.1</td>
<td>0.0</td>
<td>98.58%</td>
<td>0/633(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IBCH-3</td>
<td>KP271023.1</td>
<td>2e-174</td>
<td>99.74%</td>
<td>0/378(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IBCH-4</td>
<td>KP271023.1</td>
<td>0.0</td>
<td>100.00%</td>
<td>0/729(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IBCH-5</td>
<td>KP271023.1</td>
<td>0.0</td>
<td>99.83%</td>
<td>0/595(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IBCH-6</td>
<td>KP271023.1</td>
<td>0.0</td>
<td>100.00%</td>
<td>0/480(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IBCH-7</td>
<td>KP271023.1</td>
<td>0.0</td>
<td>99.84%</td>
<td>0/618(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IBCH-8</td>
<td>KP271023.1</td>
<td>0.0</td>
<td>99.17%</td>
<td>0/481(0%)</td>
<td>Plus/Plus</td>
</tr>
</tbody>
</table>

**Table (6): Identity of blasted isolates (IBT-1 to IBT-8) with reference sequences of highest identity percentage**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Sequence ID</th>
<th>Expect</th>
<th>Identities</th>
<th>Gaps</th>
<th>Strand</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBT-1</td>
<td>MF169214.1</td>
<td>0.0</td>
<td>99.50%</td>
<td>3/601(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IBT-2</td>
<td>KT184455.1</td>
<td>7e-139</td>
<td>100.00%</td>
<td>0/273(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IBT-3</td>
<td>KT184466.1</td>
<td>0.0</td>
<td>99.32%</td>
<td>7/1177(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IBT-4</td>
<td>KT184466.1</td>
<td>0.0</td>
<td>99.90%</td>
<td>0/979(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IBT-5</td>
<td>KT184466.1</td>
<td>0.0</td>
<td>99.90%</td>
<td>0/965(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IBT-6</td>
<td>KT184466.1</td>
<td>0.0</td>
<td>100.00%</td>
<td>0/512(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IBT-7</td>
<td>KT184466.1</td>
<td>0.0</td>
<td>99.81%</td>
<td>1/1077(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IBT-8</td>
<td>KT184466.1</td>
<td>0.0</td>
<td>100.00%</td>
<td>0/1092(0%)</td>
<td>Plus/Plus</td>
</tr>
</tbody>
</table>
allowed identification of most species to the genus level. Faster-evolving DNA regions are required to identify closely-related species\textsuperscript{[26]}. The successfully used forensically informative nucleotide sequencing analysis of the 16S rRNA mitochondrial DNA to identify before unknown biological specimens of human and animals\textsuperscript{[27]}. The mitochondrial 12S rRNA and 16S rRNA genes, including those from fish and amphibians to mammals including human beings. Therefore, universal primers were designed to amplify sequences in the fast-evolving animal mtDNA\textsuperscript{[17]}, the PCR amplifications of mitochondrial 16S rRNA followed by sequencing and analysis showed to be very efficient for identification of species origin of species\textsuperscript{[21]}. The 12S rRNA and 16S rRNA gene sequences of animals reveal the fitting level of interspecific variation but the great level of intraspecific homogeneity\textsuperscript{[28]}. The results showed no cross-reactivity of designed primer pairs and the PCR assay based on the designed primer pairs will be simple, fast, sensitive, specific, and cost-effective.

**Registration of Sequences in GenBank:**

All the 32 sequence of 16S rRNA gene were submitted to GenBank for registration. After checking and revision the following accession numbers were donated:

**16S rRNA** *homo sapiens* (Human): MN192057, MN192058, MN192059, MN192060, MN192061, MN192062, MN192063, MN192064 (Appendix 4-69 to 4-76).

**16S rRNA** *Ovis aires* (Sheep): MN173528, MN173529, MN173530, MN173531, MN173532, MN173533, MN173534, MN173535 (Appendix 4-77 to 4-84).

**16S rRNA** *Capra hircus* (Goat): MN173285, MN173286, MN173287, MN173288, MN173289, MN173290, MN173291, MN173292 (Appendix 4-85 to 4-92).

**16S rRNA** *Bos taurus* (Cow): MN197611, MN197612, MN197613, MN197614, MN197615, MN197616, MN197617, MN197618 (Appendix 4-93 to 4-100).

**Conclusion**

Sensitivity, specificity and accuracy of the designed species specific primer pairs and applicability of the designed primer pairs in forensics to investigate blood sports or evidence belonging for human, sheep, goat and cow.

**Ethical Clearance:** The project plan displayed on the scientific committee and scientific ethical committee of the department of Biology-college of science at university of Babylon and get approval.

**Source of Funding:** There is no funding source and it is completely covered by authors.

**Conflict of Interest:** There is no conflict of interest.

**References**


Curcumin Improves the Regulation of Ovarian Folliculogenesis in Culture Media with Peritoneal Fluid from Infertile Women with Endometriosis

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1Department of Obstetric and Gynecology, Faculty of Medicine, Universitas Airlangga, Surabaya (60131), Indonesia, 2Laboratory of Embriology, Faculty of Veterinary Medicine, Universitas Airlangga Surabaya, Indonesia

Abstract

Background: Peritoneal fluid (PF) from infertile women with endometriosis contains a variety of inflammatory mediators that may interfere with folliculogenesis. The aim of the study was to evaluate effects of curcumin on the regulation of ovarian folliculogenesis by evaluating Growth Differentiation Factor (GDF)-9, Kit Ligand (KitL) and Tumor Necrosis Factor (TNF)α expressions in bovine cumulus oocyte complexes (COC)s cultured with PF from infertile women with endometriosis.

Method: COCs were aspirated from antral follicles of bovine ovaries. PF was collected from infertile women with endometriosis undergoing laparoscopy for infertility evaluation. Curcumin, a strong anti-inflammatory turmeric, was added in Tissue Culture Media (TCM)199 and PF from infertile women with endometriosis for culture media. Bovine COCs were cultured into 3 groups of media: 1) TCM199, 2) TCM199+PF from infertile women with endometriosis, and 3) TCM199+PF from infertile women with endometriosis+curcumin. GDF-9, KitL and TNFα expressions were examined using immunohistochemistry technique.

Results: GDF-9 expression of bovine COCs cultured in PF from infertile women with endometriosis with curcumin addition (2.67±0.98) was found to increase compared to those cultured without curcumin (0.50±0.67) but reduced compared to the control. This result was similar to KitL expression of bovine COCs cultured with curcumin (2.67±1.23), in which it increased compared to those without curcumin (0.33±0.49) (p<0.05). A significant difference in TNFα expression was noted between groups with or without curcumin (p<0.05).

Conclusion: In the culture of PF from infertile women with endometriosis, curcumin addition may improve the regulation of ovarian folliculogenesis through the decrease of inflammation factor.

Keywords: endometriosis; curcumin; GDF9; kit ligand; TNFα

Background

Endometriosis is a condition characterized by the growth of endometri-alike tissue outside the uterus that subsequently induces a chronic inflammatory reaction. Numerous symptoms are associated with endometriosis, including dysmenorrhea, pelvic pain, dyspareunia and infertility, as well as reduced quality of life 1. The relationship between endometriosis and infertility remains unclear. One of the postulations of decreased fertility in women with endometriosis is the reduction of oocyte quality caused by abnormal folliculogenesis related to peritoneal fluid (PF) inflammation 2.

Folliculogenesis is a dynamic process marked by proliferation and differentiation of granulosa cells and maturation of oocyte. The regulation of ovarian folliculogenesis, determined by a number of growth factors and classic endocrine mechanism, provides optimal environment to produce fertilizable oocyte 3. There are two important growth factors that contribute to the regulation of ovarian folliculogenesis including the interactions between oocyte
and granulosa cells.

Those growth factors are Growth Differentiation Factor (GDF)-9 produced by oocyte, which is useful for granulosa cell proliferation and differentiation; and Kit Ligand (KitL) secreted by granulosa cells, which induces oocyte maturation. In women with endometriosis, ovaries are naturally bathed in PF that is rich in inflammatory mediators. This may cause abnormal folliculogenesis and subsequently results in infertility. The PF of women with endometriosis contains a variety of inflammatory mediators, including Tumor Necrosis Factor (TNF)α.

Curcumin, which is derived from Curcuma longa (turmeric), has a strong potential anti-inflammatory activity. It has been widely used both traditionally and scientifically to treat various conditions including inflammatory diseases, such as rheumatoid arthritis, chronic anterior uveitis and ulcerative colitis. Treatment with curcumin can reduce implant size and cell proliferation in rat endometriosis model, but the effects of curcumin on intraovarian growth factors in endometriosis and infertility remain controversial. The objectives of our study was to evaluate whether curcumin could improve the regulation of ovarian folliculogenesis in bovine cumulus oocyte complexes (COC)s by analyzing GDF-9, KitL and TNFα expressions in culture media with PF from infertile women with endometriosis.

Method

We evaluated the growth factors expression of folliculogenesis regulation on bovine COCs cultured in three different types of media. Tissue Culture Media (TCM) 199 only (group 1= control), TCM199 plus PF from infertile women with endometriosis (group 2=endometriosis), and TCM199 plus PF from infertile women with endometriosis added with curcumin (group 3=endometriosis+curcumin). The ethical board of Dr Sutomo Hospital approved the study.

Endometriosis peritoneal fluid

We obtained PF samples from women of 20 to 40 years old with endometriosis undergoing laparoscopy for infertility evaluation at Dr. Sutomo Hospital Surabaya. The diagnosis of endometriosis was made by visual inspection and peritoneal biopsy according to American Society for Reproductive Medicine criteria. We collected PF by aspiration from posterior cul-de-sac during the laparoscopic procedure. The samples were placed in a tube and centrifuged at 600 g for 10 min. The supernatants were stored at -80°C until analysis.

Curcumin

We obtained curcumin from Merck Schuchardt OHG (85662 Hohenbrunn, Germany). Curcumin (0.2 mg) was added and homogenized in 10 ml of TCM199 media and PF fluid infertile women with endometriosis (30 µl). BSA (3%) was added until the pH reached 7.4-7.8. The solution was then filtered through a 0.22-µm microfilter and 100 mL of solution was placed in a petridish for culture.

Bovine cumulus oocyte complex

COCs aspirated from antral follicles with a diameter of 3-8 mm were obtained from bovine ovaries in a slaughterhouse. The ovaries were washed and stored in 0.89% NaCl with penicillin-G (1000 IU/ml) and streptomycin sulfate (0.2 ug/l) at a temperature of 30-35°C. Before follicle aspiration, the diameter of the follicles was measured with a caliper. The COCs were aspirated using an 18-G needle connected to a 5-ml syringe containing 1 ml phosphate buffered saline (PBS) with 3% bovine serum albumin (BSA) and 50 µg/ml gentamycin. The COCs were washed 3 times successively in PBS media and one time in TCM199, placed in TCM199 media with 50 mIU/ml FSH and 50 mIU/ml LH, divided into three groups by placing them into media groups 1, 2 and 3, and then incubated at a temperature of 38°C in 5% CO2 for 24 hours. Subsequently, the COCs of each groups were fixated in a glass flask and subjected to immunohistochemical staining for GDF-9 (Biosis Antibodies Inc USA, catalog no. Bs-4720R), KitL (Abcam USA, catalog no. ab52603), and TNFα (Biosis Antibodies Inc USA, catalog no. Bs-2081R) expressions.

The three expressions were semi-quantitatively assessed according to the modified Remmele method which is the result of multiplication between the percentage score of immunoreactive cells (positive cells) with the color intensity score generated on the cell.

Statistical Analysis

Data analysis was performed using statistical software (SPSS version 17.0 for windows) Normality of variable was tested with Shapiro-Wilk test. Non-parametric test was used to detect significant differences of all variables. P < 0.05 was accepted as statistically significant.
Results

A total of 21 bovine COCs were cultured in 3 different types of media; each group contained 7 COCs. The GDF-9 expression in bovine COC was determined by dark color of immunoreactive cells on the immunohistochemical staining result. The semi-quantitative results of GDF-9 expression in control, endometriosis and endometriosis+curcumin groups were as follows: 5.83±1.58; 0.50±0.67; and 2.67±0.98, respectively. The mean expression of GDF-9 in bovine COC cultured in PF from infertile women with endometriosis+curcumin (2.67±0.98) increased compared to those cultured without curcumin (0.50±0.67) but reduced compared to the control (5.83±1.58). (p=0.46) (Figure 1).

The semi-quantitative results of KitL expression in bovine COC cultured in control, endometriosis, and endometriosis+curcumin groups were as follows: 3.92±2.02; 0.33±0.49; and 2.67±1.23, respectively. This result was similar to those of GDF-9 expression. The mean expression of KitL in bovine COC cultured in PF from infertile women with endometriosis (0.33±0.49) reduced compared to the control (3.92±2.02) and those in endometriosis+curcumin group (2.67±1.23) (p=0.001). There was also a significant difference between KitL expression in bovine COC cultured in PF from infertile women with endometriosis + curcumin and control group (p=0.035) (Figure 2).

The semi-quantitative results of TNFα expression in bovine COC cultured in control, endometriosis and endometriosis+curcumin groups were as follows: 0.00±0.00; 8.67±3.72; and 2.17±1.69, respectively. TNFα expression in bovine COC cultured in PF from infertile women with endometriosis (8.67±3.72) increased compared to those in control group (0.00±0.00), whereas TNFα expression in bovine COC cultured in PF from infertile women with endometriosis added with curcumin (2.17±1.69) reduced compared to those cultured without curcumin; however, the level increased compared to the control (p=0.001). (Figure 3).

Regression analysis revealed significant association among TNFα, GDF-9, and KitL expression cultured in PF from infertile women with endometriosis added with curcumin (p=0.00).
Discussion

The PF of infertile women with endometriosis contains a variety of inflammatory mediators and the PF component plays an essential role in the process of folliculogenesis, ovulation and fertilization. Communication between oocytes and granulosa cells occurs during folliculogenesis to produce mature and fertilizable oocytes. This process is impaired in infertile women with endometriosis. We previously reported abnormal GDF-9 and KitL expression in endometriosis. In our study GDF-9 and KitL expressions in bovine COC cultured with PF from infertile women with endometriosis were significantly reduced compared to control. It suggests that numerous inflammatory mediators in the PF of infertile women with endometriosis enters the bovine COC and further inhibits oocytes and granulosa cells activity. This results in abnormal folliculogenesis and subsequently infertility.
GDF-9 is an oocyte-specific member of the TGFβ superfamily. GDF-9-deficient female mice demonstrate a block in follicular development beyond the primary one-layer follicle stage which leads to complete infertility. In the transition to the antral stage, GDF-9 promotes follicular survival by suppressing granulosa cell apoptosis and follicular atresia. In this study, the addition of curcumin to culture media of PF from infertile women with endometriosis resulted in more improved GDF-9 expression than those without curcumin. It is possible that the anti-inflammatory effects of curcumin release oocyte suppression. By taking into account the fact that the expression of GDF-9 was still lower than the control, it indicates that it is necessary to adjust the dosage of curcumin. These findings indicate that the dose of curcumin plays a role in GDF-9 expression 16.

KitL produced by the granulosa cells in the oocyte acts by binding to c-Kit and may activate different signaling pathways. The KitL/c-Kit system regulates follicular viability, the initiation of primordial follicle growth, and oocyte and follicle development. In our study, KitL expression in bovine COC cultured in PF from infertile women with endometriosis added with curcumin significantly increased from those cultured without curcumin 17. It indicates that curcumin has positive effect on KitL expression. The changes on two growth factor expressions, GDF-9 and KitL suggest that curcumin has a repair effect on oocyte-granulosa cell interactions and the regulation of ovarian folliculogenesis18.

In order to evaluate that curcumin has repair effect on oocyte-granulosa cell interactions and the regulation of ovarian folliculogenesis, we assessed the effect of curcumin on bovine COC inflammation via TNFα expression 14. An increase in TNFα expression was noted in the PF from infertile women with endometriosis compared to that from normal women 19. In addition, we found a correlation between TNFα concentration and the degree of endometriosis severity. In fact, it has been reported that TNFα plays a role in the inflammatory process and angiogenesis, triggers follicular atresia and impairs oocyte maturation 20.

The findings of increased TNFα expressions in bovine COC cultured in PF from infertile women with endometriosis suggest that TNFα from PF enters the COC and is active. The addition of curcumin to the culture media results in an improvement as indicated by the reduced TNFα expression 16. Curcumin exhibits anti endometriosis activities by affecting MMP2 and TIMP2. It works by inhibiting cyclooxygenase2 and lipoxygenase and suppressing the activity of NFkB and pro-inflammatory gene expressions 21. Based on its mechanism of action, curcumin can suppress an inflammatory process in bovine COC cultured in PF from infertile women with endometriosis 15.

Ethical Clearance: This research process involves participants in the survey using a questionnaire that was accordant with the ethical research principle based on the regulation of research ethic committee. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, nonmaleficence, and justice.

Conflict of Interest: The authors declare that we have no conflicts of interest.

Source of Funding : All of the expenses related with this paper are paid by the authors only without any sponsor

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Correlation of Fugl-Meyer Assessment Score with Barthel Index and Functional Independence Measure in Patients with Stroke

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¹Department of Physical Medicine and Rehabilitation Faculty of Medicine Universitas Airlangga, Dr. Soetomo Teaching Hospital, Surabaya 60285, Indonesia

Abstract

Background: The prevention of stroke morbidities that is oriented to the approach of functional performance improve is expected improve the productivity of post-stroke patients. One of the tools to measure the physical performance is Fugl-Meyer Assessment (FMA) scale, while the recommended tests to measure interpedency level are Barthel Index (BI) and Functional Independence Measure (FIM).

Method: This study was conducted on 20 outpatient stroke patients in Medical Rehabilitation Installation of Dr. Soetomo General Hospital Surabaya, Indonesia. FMA, BI and FIM examination were performed in all subjects.

Results: The authors obtained the correlation coefficient value with r=0.816 and therefore a high positive correlation was found between the total score of FMA and BI, while the correlation coefficient value between the total score of FMA and FIM showed r of 0.728. Thus, the author concluded that there was a positive correlation between the total score of FMA and FIM. The sub-domains FMA-UE, FMA-LE and FMA Balance were highly correlated with BI and FIM. This implied that physical performance measured using FMA could describe the interdependency level of stroke patients in doing ADL.

Conclusion: Physical performance (based on Fugl-Meyer Assessment) was positively correlated with the interdependency level of BI and FIM of inpatient stroke patients in Medical Rehabilitation Dr. Soetomo General Hospital Surabaya.

Keywords: Fugl-Meyer Assessment, Barthel Index, Functional Independence Measure, Stroke

Introduction

Stroke is the main cause of death and disabilities in Indonesia and the world ¹. Elderly with hypertension have a greater risk for ischemic stroke ². In America, the number of post-stroke population was predicted in three millions and this number has escalated twice as much of the number of post-stroke population 25 years ago. Stroke has enormous clinical, social, and economic implications and demands a significant effort ³, the prevention of stroke morbidity that is oriented in the approach of functional performance improve is expected to improve post-stroke patients’ productivity. Stroke rehabilitation, if conducted comprehensively and accurately, will improve post-stroke patients’ life quality and productivity ⁴.

Fugl-Meyer Assessment (FMA) was first introduced in 1975 as a method for evaluating post-stroke patients’ physical conditions. Before FMA was published, almost all methods of motor recovery and stroke output evaluation were empiric-based or based on activities of daily living (ADL) evaluation. The experts of stroke rehabilitation consider FMA as one of the most
comprehensive quantitative measuring instruments and the use of it has been recommended for stroke rehabilitation clinical observation.

Functional Independence Measure (FIM) was introduced in 1984 by Granger and the joint organization of representatives of medical rehabilitation institutions in the USA (United States America). This is a common instrument used for evaluating functional conditions in various types of disease which cause disabilities and indicate how much help is needed by a disabled person in performing daily activities. FIM has subsequently developed as an instrument for clinicians to evaluate rehabilitation programs and measure the success ratio of rehabilitation programs with the costs incurred.

In 1965, Mahoney and Barthel published a scale to measure Living/ADL. The Barthel Index initially has 10 items consisting of eating, transfer, dressing up, hygiene, bathing, toileting, walking, stair climbing, and controlling micturition and defecation. Patients with the score of 100 are considered as individuals who can eat and dress independently, walk at least one block, up and down stairs, and maintain a continuous urine/aluvi. BI checks are probably the most commonly used examination in around the world to measure daily activities. Several studies have shown that this instrument has acceptable psychometric properties, including its sensitivity in measuring changes in a person’s ADL, which is a major predictor of rehabilitation outcomes, as well as BI’s examination results that are significantly associated with the patient’s medical status.

Method

This is an observational study with cross sectional study approach. This study was conducted in Medical Rehabilitation Installation of Dr. Soetomo General Hospital from August to October 2014. There were total 20 subjects that met the inclusion criteria (patients of post-thrombotic and hemorrhagic infarction stroke in outpatient unit aged of 30-70 years with hemiparesis in dominant side, stroke occurrence in ≥3 weeks, stable cooperative cardio-respiratory functions, and able to comply with simple commands) which consisted of post-ischemic and hemorrhagic stroke inpatient. The primary parameter evaluated in the subjects were Fugl-Meyer Assessment (FMA) which was divided into 6 sub-domains: FMA-UE, FMA-LE, FMA-Sensoric, FMA-balance, FMA-ROM, and FMA-joint pain. The parameter to be compared was the subjects’ level of independence by using Barthel Index questionnaire and Functional Independence Measure. All the subjects were enrolled in motor rehabilitation programs, with each subject having different program from the others. The data were analyzed using SPSS program (SPSS, Inc., Chicago, IL).

Results

Subjects general characteristics

The number of male and female subjects were 10 respectively (50%). The mean of stroke onset in the study subjects was 10.6 ±7.728 months (3-36 months). The mean of the subjects age was 55.9 ± 10.58 years, ranged between 30 to 73 years. The majority of the subjects’ dominance were right-handed. In accordance with the inclusion criteria, i.e. hemiplegia in the dominant side, most subjects (17 subjects or 85%) had right hemiparesis. The 16 subjects (80%) were generally infarction stroke patients (Table 1).

Correlation between FMA-total score and Barthel Index score

A significant correlation was found between FMA-total score and Barthel Index score with r=0.816 using Pearson’s correlation test with p value of <0.05. The graphic in XY curve also showed a correlation between the two examination instruments, in which the higher the FMA-total score was, the higher the Barthel Index score would be.

Correlation between FMA-total score and Functional Independence Measure score

A significant correlation was found between FMA-total score and Functional Independence Measure (FMI) score with correlation coefficient of r=0.728 by using Pearson’s correlation test with p >0.05.
Pearson’s correlation analysis in each sub-domain of FMA showed a correlation with FIM score with $r=0.747$ in the sub-domain FMA-UE, $r=0.719$ in the sub-domain FMA-LE and $r=0.694$ in the sub-domain FMA-balance. Similar to FMA and Barthel Index comparison, the sub-domains FMA-sensory, FMA-joint pain and FMA-ROM did not show any significant correlation with FIM score.

Table 1. Subjects’ characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Laki-laki</td>
<td>10 (50%)</td>
</tr>
<tr>
<td>Perempuan</td>
<td>10 (50%)</td>
</tr>
<tr>
<td>Age (Mean+SD)</td>
<td>55.9 ± 10.58 years</td>
</tr>
<tr>
<td>Range</td>
<td>(30–73)</td>
</tr>
<tr>
<td>Hemiparese Side</td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>17 subjects (85%)</td>
</tr>
<tr>
<td>Left</td>
<td>3 subjects (15%)</td>
</tr>
<tr>
<td>Stroke Type</td>
<td></td>
</tr>
<tr>
<td>Infarction</td>
<td>16 subjects (80%)</td>
</tr>
<tr>
<td>Hemorrhagic</td>
<td>4 subjects (20%)</td>
</tr>
<tr>
<td>Stroke Onset (Mean+SD)</td>
<td>10.6 ± 7.95 months</td>
</tr>
<tr>
<td>Range</td>
<td>(3–36)</td>
</tr>
<tr>
<td>FMA-UE (Mean+SD)</td>
<td>43.8 ± 18.38 points</td>
</tr>
<tr>
<td></td>
<td>(4 – 66)</td>
</tr>
<tr>
<td>FMA-LE (Mean+SD)</td>
<td>22.55 ± 7.69 points</td>
</tr>
<tr>
<td></td>
<td>(6 – 34)</td>
</tr>
<tr>
<td>FMA-Sensory (Mean+SD)</td>
<td>19.9 ± 3.21 points</td>
</tr>
<tr>
<td></td>
<td>(12 – 24)</td>
</tr>
<tr>
<td>FMA-Balance (Mean+SD)</td>
<td>8.25 ± 2.82 points</td>
</tr>
<tr>
<td></td>
<td>(2 – 13)</td>
</tr>
<tr>
<td>FMA-Joint pain (Mean+SD)</td>
<td>35.1 ± 6.67 points</td>
</tr>
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<td></td>
<td>(22 – 42)</td>
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<tr>
<td>FMA-ROM (Mean+SD)</td>
<td>38.15 ± 5.15 points</td>
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<tr>
<td></td>
<td>(25 – 42)</td>
</tr>
<tr>
<td>FMA-Total (Mean+SD)</td>
<td>167.5 ± 33.73 points</td>
</tr>
<tr>
<td></td>
<td>(110 – 220)</td>
</tr>
<tr>
<td>Barthel Index (Mean+SD)</td>
<td>80.75 ± 16.56 points</td>
</tr>
<tr>
<td></td>
<td>(40 – 100)</td>
</tr>
<tr>
<td>FIM (Mean+SD)</td>
<td>101.45 ± 18.38 points</td>
</tr>
<tr>
<td></td>
<td>(60 – 126)</td>
</tr>
</tbody>
</table>

*FMA = Fugl Meyer Assessment; FMA-UE: Fugl Meyer Assessment-upper extremity; FMA-LE: Fugl Meyer Assessment – Lower Extremity; FMA-ROM: Fugl Meyer Assessment- Range of Motion; FIM: Functional Independence Measure
Table 2. Correlation coefficient of FMA-total and FMA sub-domains toward Barthel Index scores using Pearson’s correlation (significant if \( p <0.05 \))

<table>
<thead>
<tr>
<th>FMA Sub-Domains</th>
<th>Correlation Coefficient (r)</th>
<th>( p ) Value (Pearson’s correlation) ( p &lt;0.05 ) significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMA – Upper Extremity</td>
<td>0.739</td>
<td>0.000*</td>
</tr>
<tr>
<td>FMA – Lower Extremity</td>
<td>0.820</td>
<td>0.000*</td>
</tr>
<tr>
<td>FMA – Balance</td>
<td>0.800</td>
<td>0.001*</td>
</tr>
<tr>
<td>FMA – Sensorik</td>
<td>0.422</td>
<td>0.064</td>
</tr>
<tr>
<td>FMA – Joint pain</td>
<td>0.378</td>
<td>0.100</td>
</tr>
<tr>
<td>FMA – Range of Motion</td>
<td>0.291</td>
<td>0.212</td>
</tr>
<tr>
<td>FMA – Total</td>
<td>0.816</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

Table 3. Correlation coefficient of FMA-total and FMA sub-domains towards FIM scores using Pearson’s correlation (significant if \( p <0.05 \))

<table>
<thead>
<tr>
<th>FMA Sub-Domains</th>
<th>Correlation Coefficient (r)</th>
<th>( p ) Value (Pearson’s) ( P &lt;0.05 ) significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMA – Upper Extremity</td>
<td>0.728</td>
<td>0.000*</td>
</tr>
<tr>
<td>FMA – Lower Extremity</td>
<td>0.747</td>
<td>0.000*</td>
</tr>
<tr>
<td>FMA – Balance</td>
<td>0.694</td>
<td>0.001*</td>
</tr>
<tr>
<td>FMA – Sensorik</td>
<td>0.267</td>
<td>0.256</td>
</tr>
<tr>
<td>FMA – Joint pain</td>
<td>0.283</td>
<td>0.226</td>
</tr>
<tr>
<td>FMA – Range of Motion</td>
<td>0.116</td>
<td>0.625</td>
</tr>
<tr>
<td>FMA – Total</td>
<td>0.728</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

Discussion

The result of this study showed that most subjects (10 subjects or 50%) had significant motor impairment, while the other subjects had slight and severe impairment with 6 (30%) and 4 (20%) subjects respectively. A study by Duncan in 1992 with the subjects consisted of 104 stroke patients reported that 37 subjects (35.6%) had slight motor impairment, 2 subjects (21.2%) had moderate motor impairment, 13 subjects (12.5%) had significant motor impairment and 32 subjects (30.8%) had severe motor impairment ⑨.

In this study, the obtained mean of FMA-UE was 43.08±18.38 (4-66), while the mean of FMA-LE score was 22.55±7.69 (6-34). The mean of FMA-sensory score was 19.9±3.21 (12-24). This was in line with the results of a study by Sullivan et al. conducted in 15 stroke patients in which the obtained mean of FMA-UE was 30.40 (5-63) and FMA-LE motor score mean was 23.10 (8-33), whereas the mean of FMA-sensory total score was 18.70 (2-24). This implied that the mean of FMA-LE motor score was higher than that of FMA-UE motor score if reviewed from each instruction item’s score and sensory total score which were not much different from
the results from Sullivan et al. 10.

The mean of FMA-balance score was 8.25±2.82 with the lowest score being 2 and the highest score 14. The score of FMA-balance was lower than that in the study by de Oliveira et al. in Physiotherapy and Occupational Therapy Ambulatory of the University Hospital-UNICAMP, Brazil, in which the obtained FMA-balance of the 20 stroke patients was 10.80±1.24 7.

The mean value of FMA-Total in this study was 167.5 ± 33.73 (110-220). The mean FMA-total value was consistent with research conducted by de Oliveira, i.e. 170.50±31.91. The mean FMA-total in this study indicated that the functional state of the subjects was in a phase where there a controlled movement occurred outside of the synergistic pattern of the limbs and the spasticity level that has begun to decline 7.

Barthel Index score mean obtained was 80.75±16.56 points and ranged between 40-100 points. This was slightly different from the results obtained by de Oliveira et al in which the authors also evaluate the correlation between FMA and Barthel Index. The mean of Barthel Index score in that study was 90.75±5.45 7.

The mean of the subjects’ FIM score was 101.45±18.38 points and ranged from between 60-126 points. This indicated the subjects’ state where they could perform daily activities although the caregivers’ supervision was still required in each FIM measurement. The mean of FIM score here was higher compared to the results from Ravaud J.F. et al who tested the FIM constructive validity as a rehabilitation instrument. The mean of Barthel Index score in that study was 90.75±5.45 7.

This study’s results suggested that FMA-total score was significantly correlated with Barthel Index with the correlation coefficient of r=0.816. The correlation coefficient indicated a strong correlation between FMA score and BI score. The results of this study showed a higher correlation coefficient value compared to that of the study conducted by Oliveira et al. who obtained a statistically significant correlation coefficient (P <0.05) of r = 0.597. This indicated that the level of physical performance measured based on the FMA value affected a person’s level of independence in performing daily activities (ADL/Activity Daily Living). The high correlation coefficient in the study implied that FMA score could be used as a reference to measure a stroke patient’s independence level 7.

The results of this study also indicate that the sub-domains FMA-UE, FMA-LE and FAM-balance had statistically strong correlation with Barthel Index score. This suggested that motor function recovery (AGA and AGB) in stroke patients along with the post-stroke balance level were correlated in improving a stroke patient’s independence. This was also similar to the study by de Oliveira where a significant correlation was found between the sub-domain motor total and Barthel Index score with r=0.597. In addition, Ferrucci et al. also reported a quite statistically significant correlation between FMA score and Barthel Index score.

AGA was known to have a bigger role in self-care while AGB affected mobility. However, the correlation coefficient in this study was higher in FMA-LE than in FMA-UE toward Barthel Index. The authors assumed that this was because in performing self-care activities, the subjects have done substitutions in the non-dominant arm that did not impacted by hemiparese. This was stated by Olsen et al. who assumed that a compensatory mechanism has occurred as a strategy in functional performance. The mobility components were considered as the components causing FMA-LE and FMA-balance to have a statistically significant correlation with Barthel Index. Correlation between balance and functional performance has often been documented in hemiparese patients, in which a posture impairment was found that was related to standing and sitting balance and this would lead to impairments in functional performance related to mobilization 7,12.

Other results showed that FMA total score had statistically correlation with FIM score with r=0.728. FIM was the more current instrument and had a rapid popularity increase. Aside of measuring ADL, this examination also had social and communication cognitive items. This instrument was considered more quantitative compared to Bathel Index since the score of each FIM item was ranged between 1-7 and thus the score of each item would be more specific.

A lot number of evidences from several validation studies confirmed that the FMA score could represent the independence level or disability level of stroke patients. In this study, the scores were found in FMA-UE, FMA-LE and FMA-balance that were significantly correlated with FIM score, with correlation coefficient of 0.728, 0.747 and 0.694 respectively. All FMA components were highly correlated with FIM. This was understandable since FIM items required functional
motor strength and balance for independent functional performance. However, the correlation coefficient of FMA-BI was higher than that of FMA-FIM. It was caused by the cognitive and communication components in FIM were not empirically correlated with motor performance or balance. However, cognitive and communication components had lower score proportion compared to self-care, sphincter control, mobility and locomosi in which then correlation between FMA-total and FIM remained found despite the correlation coefficient being lower than that of FMA-total and BI.

**Conclusion**

A high positive correlation was found between functional performances measured using Fugl-Meyer Assessment and interdependency levels in performing daily activities measured using Functional Independent Measure (FIM) score in stroke patients with dominant-side hemiparese undergoing the rehabilitation programs in Medical Rehabilitation Hospital.

**Conflict of Interest** : There is no conflict of interest

**Source of Funding** : This study is self funded

**Ethical Clearance** : This study was approved by Ethical Commission of Health Research Faculty of Medicine University of Airlangga

**References**


Determination of Priority Elements of Vigilance in the Use of Pesticides based on Difficulty and Usefulness (A Supporting Study for Law and Policy in Health)

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Abstract

The use of pesticides is still a problem for public health, therefore we need a regulation by the authorities, which can lead to the behavior of using pesticides correctly. This is part of the area of law and health policy. Pesticide poisoning can cause breathing difficulties, headaches, neurological or psychological effects, and skin irritation and mucous membranes. The purpose of this study is to provide an overview of some elements of vigilance in the use of chemical pesticides for farmers. The variables studied were difficulty and usefulness with eight elements, namely the use of personal protective equipment (PPE), pesticide storage, pesticide use procedures, use of pesticide doses, duration and frequency of pesticide spraying, maintaining equipment cleanliness, pesticide spraying according to wind direction and time of pesticide spraying. The sample size of this study was 100 respondents from the community who knew about the use of pesticides throughout Indonesia. The research instrument used was a Google form questionnaire sent to respondents via Whatsapp social media. The data that has been collected is analyzed descriptively to get an idea of which elements are the priorities for improvement. Referring data analysis result, the elements with high difficulty and usefulness together were use of pesticide doses and procedures for using pesticides. Thus, the two elements selected as priority elements will be fixed first. It is suggested that the pesticide control policy makers can develop regulations by prioritizing the two main elements, without ignoring the other six elements. In addition, it is also recommended that other control efforts be made that can be recommended to related parties in the area of law and health policy, related to the prevention of health problems caused by the use of pesticides.

Keywords: health law and policy, pesticide; farmer; difficulty; usefulness

Introduction

The use of pesticides is still a problem for public health, therefore we need a regulation by the authorities, which can lead to the behavior of using pesticides correctly. This is part of the area of law and health policy. With formal regulation, efforts to control the use of pesticides can be better regulated, so that the negative impacts that occur can be minimized.

Pesticides have a major role in increasing agricultural production. Based on experience in Latin America, the use of pesticides can increase cocoa production by 40%. In Pakistan, pesticides help increase sugarcane production by 33%, and based on FAO records the use of pesticides can save cotton crops up to 50%.(1)

Many research results show a relationship between the use of pesticides with health problems in workers. According to WHO, intentional or unintentional pesticide poisoning is a serious problem in agricultural communities in poor and developing countries. It is estimated that around 250,000 deaths occur due to pesticide poisoning each year.(2)
Symptoms of poisoning arising from the use of pesticides include difficulty breathing, headaches, neurological or psychological effects, and skin irritation and mucous membranes. Manifestation of these effects depends on the type of pesticide and the level and duration of exposure.\(^3\)

Advanced health problems arising from exposure to pesticides are mutagenic, carcinogenic, endocrine disruptors, reproductive and neurotoxic disorders.\(^4\) Pesticides can enter the body through digestion, inhalation and through the surface of the skin that is not protected.\(^5\) The presence of pesticide exposure in the body can be determined by checking the activity of the cholinesterase enzyme. Organophosphate pesticides work by inhibiting the activity of the cholinesterase enzyme so that acetylchholin cannot be hydrolyzed, so that the amount becomes excessive, which in turn can result in continuous stimulation of the muscarinic and nicotinic nerves.\(^6\)

Horticultural crop farmers are one of the populations at risk for pesticide poisoning, with long-term negative impacts. The risk of this exposure is related to their involvement in activities in agriculture, such as spraying, preparing equipment for spraying, mixing pesticides, washing equipment and clothing used when spraying, removing weeds from plants, removing pests, watering plants and harvesting.

In Indonesia, the proportion of pesticide use or storage in the home is 20%. This shows the high risk of exposure to pesticides, not only in rice fields but also in the household environment.\(^7\) Farmers who use chemical pesticides in Indonesia, most do not pay attention to pesticide use standards, although many have received counseling from the agriculture and health offices in pesticide prevention and control programs. So far, risky behavior carried out by farmers in the use of pesticides has not decreased, therefore, research needs to be carried out that aims to identify and provide an overview of the selection of elements of behavior of farmers who are very risky, to prioritize which elements need to be dealt with quickly quickly and right.

**Method**

This research was a descriptive study conducted in 2019, involving the general public who know about the use of chemical pesticides in Indonesia as research respondents, with a sample size of 100 respondents.

The research variables measured were difficulty and usefulness\(^{(8),(9)}\) of 8 elements of risk behavior consisting of; 1) personal protective equipment (PPE), 2) storage of pesticides, 3) procedures for using pesticides, 4) use of pesticide doses, 5) duration and frequency of spraying pesticides, 6) maintain cleanliness of equipment, 7) spraying pesticides in the direction of the wind, 8) time spraying pesticides. The selection of these elements was based on references written by Djojosumarto, titled “Pesticides and Their Applications”\(^{(10)}\). In this questionnaire, difficulty was scored with a negative symbol (0 to -10), so the higher the difficulty of an element, the score for that element increasingly negative. Usefulness was scored with positive symbols (0 to 10), so the higher the usefulness of an element, the more positive the score for that element was.

The research instrument used was a Google form questionnaire sent to respondents via Whatsapp social media. The categorical data that has been collected was analyzed descriptively in the form of frequency\(^{(11)}\) to get an idea of which elements were the priorities for improvement.

<table>
<thead>
<tr>
<th>Difficulty</th>
<th>Elements</th>
<th>Usefulness</th>
</tr>
</thead>
<tbody>
<tr>
<td>High -10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 Low</td>
<td>Personal protective equipment (PPE)</td>
<td>Low 0 1 2 3 4 5 6 7 8 9 10 High</td>
</tr>
<tr>
<td>High -10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 Low</td>
<td>Storage of pesticides</td>
<td>Low 0 1 2 3 4 5 6 7 8 9 10 High</td>
</tr>
<tr>
<td>High -10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 Low</td>
<td>Procedure for using pesticides</td>
<td>Low 0 1 2 3 4 5 6 7 8 9 10 High</td>
</tr>
<tr>
<td>High -10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 Low</td>
<td>Use of pesticide doses</td>
<td>Low 0 1 2 3 4 5 6 7 8 9 10 High</td>
</tr>
<tr>
<td>High -10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 Low</td>
<td>Duration and frequency of spraying pesticides</td>
<td>Low 0 1 2 3 4 5 6 7 8 9 10 High</td>
</tr>
<tr>
<td>High -10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 Low</td>
<td>Maintain cleanliness of equipment</td>
<td>Low 0 1 2 3 4 5 6 7 8 9 10 High</td>
</tr>
<tr>
<td>High -10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 Low</td>
<td>Spraying pesticides in the direction of the wind</td>
<td>Low 0 1 2 3 4 5 6 7 8 9 10 High</td>
</tr>
<tr>
<td>High -10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 Low</td>
<td>Time spraying pesticides</td>
<td>Low 0 1 2 3 4 5 6 7 8 9 10 High</td>
</tr>
</tbody>
</table>

Figure 1. The research instrument
Findings

The results of descriptive data analysis are shown in Table 1.

**Table 1. The distribution of difficulty**

<table>
<thead>
<tr>
<th>Variable name</th>
<th>Difficulty</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Easy</td>
</tr>
<tr>
<td>Personal protective equipment (PPE)</td>
<td>32</td>
</tr>
<tr>
<td>Storage of pesticides</td>
<td>37</td>
</tr>
<tr>
<td>Procedure for using pesticides</td>
<td>27</td>
</tr>
<tr>
<td>Use of pesticide doses</td>
<td>24</td>
</tr>
<tr>
<td>Duration and frequency of spraying pesticides</td>
<td>26</td>
</tr>
<tr>
<td>Maintain cleanliness of equipment</td>
<td>32</td>
</tr>
<tr>
<td>Spraying pesticides in the direction of the wind</td>
<td>36</td>
</tr>
<tr>
<td>Time spraying pesticides</td>
<td>33</td>
</tr>
</tbody>
</table>

Regarding difficulty, elements with a relatively high level of difficulty (score -6 to -10) were use of pesticide doses, duration and frequency of spraying pesticides, and procedures for using pesticides.

**Table 2. The distribution of usefulness**

<table>
<thead>
<tr>
<th>Variable name</th>
<th>Usefulness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Useful</td>
</tr>
<tr>
<td>Personal protective equipment (PPE)</td>
<td>22</td>
</tr>
<tr>
<td>Storage of pesticides</td>
<td>26</td>
</tr>
<tr>
<td>Procedure for using pesticides</td>
<td>38</td>
</tr>
<tr>
<td>Use of pesticide doses</td>
<td>29</td>
</tr>
<tr>
<td>Duration and frequency of spraying pesticides</td>
<td>15</td>
</tr>
<tr>
<td>Maintain cleanliness of equipment</td>
<td>26</td>
</tr>
<tr>
<td>Spraying pesticides in the direction of the wind</td>
<td>28</td>
</tr>
<tr>
<td>Time spraying pesticides</td>
<td>34</td>
</tr>
</tbody>
</table>

Regarding usefulness, elements with a relatively high level of usefulness (score 6 to 10) were procedures for using pesticides, time spraying pesticides, and use of pesticide doses.

Referring to Table 1 and Table 2, it can be seen that the elements with high difficulty and usefulness together were use of pesticide doses and procedures for using pesticides. Thus, the two elements selected as priority elements will be fixed first.
Discussion

Based on the results of the study, it is known that there are 2 elements that are prioritized for improvement, referring to the difficulty and usefulness according to public perception. The two elements are:

1) Use of pesticide doses
2) Procedures for using pesticides

The two elements above were chosen as elements to be addressed first, but that does not mean ignoring the other six elements. Selection as an element of priority is limited to the issue of time urgency. Because these two elements have the highest level of difficulty to be changed for the better and have the highest level of benefit for the community, both of them are chosen to be addressed first.

The use of difficulty and usefulness as a priority determination refers to the findings pioneered by Nugroho et al.\(^1\)\(^2\) which was originally applied to research on the elements of e-learning in the health field, which has been realized in two studies namely the use of difficulty and usefulness without weighting and weighting. In both studies it is recommended that this priority setting method can be applied to various fields of science. Thus, in this study also applied the use of difficulty and usefulness as a method of selecting elements that are prioritized to be addressed first in the framework of the program of vigilance against the behavior of the use of wrong pesticides, which could adversely affect health.

However, in this study, only the main elements were selected with high difficulty and usefulness values together, based on descriptive data analysis in the form of frequency. Meanwhile, Nugroho et al.\(^1\)\(^2\)\(^3\) in both researches, applying in different ways using numerical data analysis in the form of mean scores for each element, and presented in the form of Difficulty-Usefulness Pyramid (DUP).

Conclusion

Based on the results of data analysis, it can be concluded that based on difficulty and usefulness, there are two main elements that are prioritized to be addressed first, namely use of pesticide doses and procedures for using pesticides.

Based on the conclusions above, it is suggested that the pesticide control policy makers can develop regulations by prioritizing the two main elements, without ignoring the other six elements. In addition, it is also recommended that other control efforts be made that can be recommended to related parties in the area of law and health policy, related to the prevention of health problems caused by the use of pesticides.

Conflict of Interest: No

Source of Funding: Authors

Ethical Clearance: Yes

References

9. Nugroho HSW, Handoyo, Prayitno H, Budiono A. Sort Elements Based on Priority, in order to


The Association Between Myoglobin, Troponin I, Hfabp and Nt-Probnp Levels with Acute Myocardial Infarction in Patients with Acute Coronary Syndrome

Ime Fransiska Adidharma1, Jusak Nugraha1, Muhammad Aminuddin1

1Department of Clinical Pathology Faculty of Medicine Universitas Airlangga Dr. Soetomo Teaching Hospital Surabaya60285, Indonesia

Abstract

Introduction: Myocardial infarction is one of the five major manifestations of coronary heart disease. Myoglobin heart markers, Isoenzyme Creatine Kinase-MB (CK-MB), cardiac Troponin I (cTnI) or Troponin T (cTnT), Heart type fatty acid-binding protein (HFABP) and NT-proBNP are currently used in assisting the diagnosis of acute myocardial infarction. The study aimed to analyze the association of cardiac marker examination results of myoglobin, cTnI, HFABP and NT-proBNP with AMI occurrence on examination I and examination II.

Method: This study enrolled 33 patients in Installation of Emergency Care with suspected acute coronary syndrome, from March to August 2013. Serum samples were taken including cTnT, myoglobin, cTnI, HFABP and NT-proBNP. Then, it was further analyzed.

Results: There was an association between myoglobin, cTnI, HFABP, and NT-proBNP with AMI events. Myoglobin and HFABP were early markers for the diagnosis of AMI, the mean levels of both markers was higher at the first examination (<1 hour admitted patients) when compared with 6 hours later after being tested. Essentially, the diagnostic value of both did not differ, only HFABP was more specific than myoglobin. Troponin I showed to be the best choice in the diagnosis of AMI because of the high sensitivity and specificity.

Conclusion: An association between myoglobin, cTnI, HFABP, and NT-proBNP with AMI events was found. Myoglobin, cTnI, HFABP can be used in diagnosing AMI patients, whereas NT-proBNP is not recommended for the diagnosis of AMI due to its low specificity.

Keywords: AMI, ACS, myoglobin, cTnI, HFABP, NT-proBNP

Introduction

Cardiovascular disease is a global public health problem, contributing to 30% of global deaths and 10% of global diseases.1

Acute myocardial infarction is part of the spectrum of acute coronary syndrome. Acute coronary syndromes include Unstable angina (UA), Non-ST-elevation myocardial infarction (NSTEMI), and ST-elevation myocardial infarction (STEMI).1,2

World Health Organization (WHO) also shows that deaths from ischemic heart disease ranked at 12.2% in 2004 and 14.2% in 2008.3 Indonesia’s health profile in 2009 expended by the Ministry of Health of Indonesia shows that in 2008 vascular system disease was highest among 11.06% of all causes of death in the hospital.4

Early diagnosis of myocardial infarction is still a problem because not all patients who later prove to be suffering from AMI come with clear diagnostic criteria, especially in the early hours. Cardiac serial examination

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is now universally accepted as an important determinant in the diagnosis of acute myocardial infarction. Appropriate and rapid method of examination for early diagnosis of myocardial infarction is essential.5-9

Myoglobin heart markers, Isoenzyme Creatine Kinase-MB (CK-MB), cardiac Troponin I (cTnI) or Troponin T (cTnT), Heart type fatty acid-binding protein (HFABP) and NT-proBNP are currently used in assisting the diagnosis of acute myocardial infarction.8

Heart type fatty acid-binding protein (HFABP) is a low-BM-dissolved protein, which is widely present in the myocardial cell cytoplasm. HFABP is a rapid biological marker in the circulation after myocardial injury.10-11 B-type natriuretic peptide is a hormone that is structurally included in the group of natriuretic peptide hormones associated with renal and cardiovascular function. Increased levels of natriuretic peptide, especially NT-proBNP, are also found in post-myocardial infarction, which may be caused by localized infarct strains and are associated with activation of the neurohormonal system.12-13 This study aimed to analyze the association of cardiac marker examination results of myoglobin, cTnI, HFABP, NT-proBNP with the incidence of AMI in patients suspected of acute coronary syndrome who came to emergency unit of Dr. Soetomo General Hospital on examination I (<1 hour on admission) and examination II (6 hours after admission), and compare the sensitivity and specificity of the heart marker.

Method

This study was an observational analytic study using a cross-sectional study design. The study was implemented in Emergency Department Dr. Soetomo Teaching Hospital, Surabaya Indonesia. The samples were 50 patients who came to emergency unit Dr. Soetomo Teaching Hospital within 1 month of suspected ACS, suspected acute myocardial infarction fulfilled the sample criteria.

The inclusion criteria in the study included patients with chest pain complaints with suspected ACS, willing to participate in the research and signed informed consent and had a complete medical record. On the other hand, the exclusion criteria included patients with serum creatinine >2mg/dL, sepsis patients, cirrhosis hepatis and acute stroke. The flow of the study began when emergency unit patients were diagnosed with acute syndrome syndrome, and matched with inclusion criteria. If it was appropriate, it was continued for the next stage of anamensis, physical examination, ECG, cTnT examination, myoglobin, cTnI, HFABP and NT-proBNP. Examination I (<1 hour of patient admission) and examination II (6 hours after admission) were conducted. The subjects were distinguished into AMI patients and non AMI patients. Afterwards, the data collection and data processing were conducted using statistical analysis.

All data was obtained and inserted into the computer, then then processed statistically. The results were presented in tables or graphs. The diagnostic test criteria included sensitivity, specificity, negative and positive predictive value calculated using 2x2 tables based on 95% CI. An association analysis between the levels of myoglobin, troponin I, HFABP and NT-proBNP with AMI events was analyzed by Chi-square test.

Results

The sample characteristics showed in table 1.

<table>
<thead>
<tr>
<th>Characteristics of research sample and characteristics of AMI patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics of samples</td>
</tr>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>20 – 29 years old</td>
</tr>
<tr>
<td>30 – 39 years old</td>
</tr>
<tr>
<td>40 – 49 years old</td>
</tr>
<tr>
<td>50 – 59 years old</td>
</tr>
</tbody>
</table>
The results of cTnT examination showed that there were 3 patients who were at the beginning of the negative examination and on the 6th positive check. The mean cTnI, HFABP and NT-proBNP levels were higher in the 6 hours of patient admission. The mean myoglobin levels was higher at examination 1 (<1 hour of admission) (Table 2). HFABP levels in AMI (STEMI and NSTEMI) patients were higher than in non-IMA patients, with higher HFABP levels of STEMI patients than in NSTEMI patients.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>AMI</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>60 – 69 years old</td>
<td>3 (9.1%)</td>
<td></td>
</tr>
<tr>
<td>70 – 79 years old</td>
<td>5 (15.2%)</td>
<td></td>
</tr>
<tr>
<td>AMI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>STEMI</td>
<td>16 (84.2%)</td>
<td></td>
</tr>
<tr>
<td>NSTEMI</td>
<td>3 (15.8%)</td>
<td></td>
</tr>
<tr>
<td>Non AMI</td>
<td>14 (42.4%)</td>
<td></td>
</tr>
<tr>
<td>Characteristic of AMI patients</td>
<td>N(%)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>12 (63.2%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>7 (36.8%)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 – 29 years old</td>
<td>1 (5.3%)</td>
<td></td>
</tr>
<tr>
<td>30 – 39 years old</td>
<td>1 (5.3%)</td>
<td></td>
</tr>
<tr>
<td>40 – 49 years old</td>
<td>4 (21%)</td>
<td></td>
</tr>
<tr>
<td>50 – 59 years old</td>
<td>9 (47.4%)</td>
<td></td>
</tr>
<tr>
<td>60 – 69 years old</td>
<td>2 (10.5%)</td>
<td></td>
</tr>
<tr>
<td>70 – 79 years old</td>
<td>2 (10.5%)</td>
<td></td>
</tr>
<tr>
<td>History</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>12 (63.2%)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>7 (36.8%)</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>13 (68.4%)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>6 (31.6%)</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>11 (57.9%)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>8 (42.1%)</td>
<td></td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 200 mg/dL</td>
<td>8 (42.1%)</td>
<td></td>
</tr>
<tr>
<td>&lt; 200 mg/dL</td>
<td>11 (57.9%)</td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 150 mg/dL</td>
<td>12 (63.2%)</td>
<td></td>
</tr>
<tr>
<td>&lt; 150 mg/dL</td>
<td>7 (36.8%)</td>
<td></td>
</tr>
<tr>
<td>HDL Cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 40 mg/dL</td>
<td>2 (10.5%)</td>
<td></td>
</tr>
<tr>
<td>&lt; 40 mg/dL</td>
<td>17 (89.5%)</td>
<td></td>
</tr>
<tr>
<td>LDL Cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 100 mg/dL</td>
<td>13 (68.4%)</td>
<td></td>
</tr>
<tr>
<td>&lt; 100 mg/dL</td>
<td>6 (31.6%)</td>
<td></td>
</tr>
</tbody>
</table>

Cont... Table 1 Characteristics of Research Sample and Characteristics of AMI Patients
Table 2 The mean levels of examination results and diagnostic value

<table>
<thead>
<tr>
<th>Level of examination results (n=33)</th>
<th>Mean</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Positive prediction value (%)</th>
<th>Negative prediction value (%)</th>
<th>Positive likelihood ratio</th>
<th>Negative likelihood ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myoglobin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myoglobin I (≤ 1 hour)</td>
<td>146.40±79±146</td>
<td>78.9% (0.57-0.91)</td>
<td>92.9% (0.69-1.0)</td>
<td>93.8% (0.72-9.9)</td>
<td>76.5% (0.53-0.91)</td>
<td>11.052 (1.95-9.23)</td>
<td>0.23 (0.08-0.59)</td>
</tr>
<tr>
<td>Myoglobin II (6 hours)</td>
<td>139.88±79±123</td>
<td>78.9% (0.57-0.91)</td>
<td>92.9% (0.69-1.0)</td>
<td>93.8% (0.72-9.9)</td>
<td>76.5% (0.53-0.91)</td>
<td>11.052 (1.95-9.23)</td>
<td>0.23 (0.08-0.59)</td>
</tr>
<tr>
<td>cTnI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cTnI I (≤ 1 hour)</td>
<td>3.99±6.72</td>
<td>94.7% (0.75-0.99)</td>
<td>100% (0.78-1.0)</td>
<td>100% (0.7-0.99)</td>
<td>93.3% (0.7-0.99)</td>
<td>9.22 (0.0-9.23)</td>
<td>0.05 (0.01-0.37)</td>
</tr>
<tr>
<td>cTnI II (6 hours)</td>
<td>4.92±6.96</td>
<td>100% (0.83-1)</td>
<td>100% (0.78-1.0)</td>
<td>100% (0.83-1)</td>
<td>76.5% (0.78-1)</td>
<td>9.2 (0.0-9.23)</td>
<td>0.0 (0.0-0.0)</td>
</tr>
<tr>
<td>HFABP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HFABP I (≤ 1 hour)</td>
<td>32.63±43.19</td>
<td>78.9% (0.57-0.91)</td>
<td>100% (0.78-1.0)</td>
<td>100% (0.8-1.0)</td>
<td>77.8% (0.55-0.91)</td>
<td>9.2 (1.95-9.23)</td>
<td>0.21 (0.08-0.56)</td>
</tr>
<tr>
<td>HFABP II (6 hours)</td>
<td>33.37±42.78</td>
<td>72.7% (0.51-0.88)</td>
<td>100% (0.78-1.0)</td>
<td>100% (0.8-1.0)</td>
<td>73.7% (0.51-0.88)</td>
<td>9.2 (1.95-9.23)</td>
<td>0.26 (0.11-0.63)</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT-proBNP I (≤ 1 hour)</td>
<td>1656.50±3537</td>
<td>78.9% (0.57-0.91)</td>
<td>50% (0.27-73)</td>
<td>68.2% (0.47-84)</td>
<td>63.6% (0.35-0.85)</td>
<td>1.58 (0.84-2.96)</td>
<td>0.42 (0.15-1.15)</td>
</tr>
<tr>
<td>NT-proBNP II (6 hours)</td>
<td>2301.46±3639</td>
<td>89.5% (0.89-0.97)</td>
<td>50% (0.27-73)</td>
<td>70.8% (0.51-0.85)</td>
<td>77.8% (0.45-0.94)</td>
<td>1.79 (0.92-3.40)</td>
<td>0.21 (0.06-0.8)</td>
</tr>
</tbody>
</table>

The suitability of myoglobin, cTnI, HFABP, NT-proBNP towards cTnT results showed in Table 3.
Table 3 Suitability of myoglobin, cTnI, HFABP, NT-proBNP towards cTnT results

<table>
<thead>
<tr>
<th>Examination results I (≤ 1 jam)</th>
<th>cTnT</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Myoglobin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>cTnI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>HFABP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>9</td>
</tr>
</tbody>
</table>

Result of Examination I (6 jam)

| Myoglobin                     |     |       | 0.001 |
| Positive                      | 15  | 1     |       |
| Negative                      | 3   | 14    |       |
| cTnI                          |     |       | 0.001 |
| Positive                      | 18  | 1     |       |
| Negative                      | 0   | 14    |       |
| HFABP                         |     |       | 0.001 |
| Positive                      | 14  | 0     |       |
| Negative                      | 4   | 15    |       |
| NT-proBNP                     |     |       | 0.022 |
| Positive                      | 16  | 8     |       |
| Negative                      | 2   | 7     |       |

The results of conformity of myoglobin, cTnI, HFABP, NT-proBNP results towards the golden standard in AMI patients showed in Table 4.
**Table 4 Conformity of myoglobin, cTnI, HFABP, NT-proBNP results towards the golden standard in AMI patients**

<table>
<thead>
<tr>
<th>Examination result I (≤ 1 hour)</th>
<th>Golden standard</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Myoglobin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>cTnI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>HFABP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
<td>7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Examination result I (6 hours)</th>
<th>Golden standard</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Myoglobin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>cTnI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>HFABP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>7</td>
</tr>
</tbody>
</table>

**Analysis of associations of myoglobin, cTnI, HFABP and NT-proBNP with AMI incidence**

Based on Fisher’s exact test, in examination I, (<1 hour of MRS patient) the value of p was 0.005 which meant less than α = 0.05. It can be concluded that there was significant association between myoglobin and AMI event. Fisher’s exact test result on examination 6 hours after patient admission showed that p = 0.005 which meant less than α = 0.05. Thus, it can be concluded that there was significant association between myoglobin and AMI event. Fisher’s exact test result, on examination I
(<1 hour of patient MRS), the p value was 0.000 which meant less than α = 0.05. Thus, it can be concluded that there was a significant association between cTnI with AMI event. Fisher’s exact test result on examination 6 hours after patient admission showed that p = 0.000 which meant less than α = 0.05. Therefore, it can be concluded that there was significant association between cTnI and AMI event.

Based on Fisher’s exact test, on examination I (<1 hour of patient admission) the value of p was 0.002 which meant less than α = 0.05. Thus, it can be concluded that there was a significant association between HFABP and AMI event. Fisher’s exact test result on examination 6 hours after patient admission showed that p = 0.002 which meant less than α = 0.05. Therefore, it can be concluded that there was a significant association between HFABP and AMI event. Result of Fisher’s exact test test, on examination I (<1 hour of patient admission) the p value was 0.026 which meant less than α = 0.05. Thus, it can be concluded that there was significant association between NT-proBNP and AMI event. Fisher’s exact test result on examination 6 hours after patient admission showed that p = 0.017 which meant less than α = 0.05. Therefore, it can be concluded that there was significant association between NT-proBNP and AMI event.

Discussion

An individual is very susceptible to coronary atherosclerosis along with the aging process. The incidence of AMI increased fivefold at the age of 40 to 60 years old. It is associated with increasing age (especially in the menopausal phase), where there is a decrease in endogenous estrogen levels. Most AMI patients suffer from dyslipidemia. The study of multiple risk factor intervention trials in 356,222 subjects showed that cholesterol levels rise in proportion to the increase of AMI attacks. Increased LDL and HDL decline are important risk factors for AMI. Any decrease of 4 mg% of HDL will increase AMI risk by about 10%. The National Academy of Clinical Biochemistry (NACB) recommends serial examination and the use of 2 cardiac markers of myocardial necrosis: early and slow-emerging cardiac markers.

Myoglobin examination showed if the highest results in the STEMI group, it corresponds to several literatures that stated that the increase in myoglobin in the blood occurs 1-4 hours after damage of the heart muscle tissue or skeletal muscle, reaches peak within 6-7 hours after myocardial infarction, and returns to normal within 24 hours. Because myoglobin is an early heart marker, it is necessary to check with other cardiac markers.

Based on the results of research on the highest average cTnI examination in the STEMI group on the second examination, it is consistent with the literature which states that troponin I begins to increase 3 to 5 hours after myocardial injury, peaking at 14 to 18 hours and keep increasing for 5 to 7 days. Increased concentrations of BNP and NT-proBNP during the arrival in hospital under conditions of acute coronary syndrome are associated with poor prognosis, including increased mortality, congestive heart failure, and recurrent ischemic events.

Suitability of myoglobin, cTnI, HFAB, NT-proBNP results against gold standard in AMI patients. Troponin I is an ideal AMI biochemical marker because of its sensitivity and specificity. These biochemical markers are not affected by skeletal muscle disease, skeletal muscle trauma, renal disease or surgery. A multifaceted mobile technology-supported primary health care intervention was associated with greater use of preventive CVD medication and lower BP levels among high-risk individuals in a rural Indonesian population. Conclusions High cardiovascular risk is common among Indonesian adults aged 40 years, and rates of preventive treatment are low.

Conclusion

It can be concluded that there was an association between myoglobin, cTnI, HFAB and NT-proBNP with the incidence of AMI. The emergence of biomarkers does not occur at the same time, therefore serial cardiovascular examination and use of 2 cardiac markers in diagnosing AMI are required, considering the presence of early marker and a marker that appears more slowly, and in view of the different sensitivity and specificity of each cardiac marker.

Ethical Clearance: This research is approved by Ethical Clearance from Universitas Airlangga dan Dr Soetomo Teaching Hospital

Conflict of Interest: There is no conflict of interest reported from this research

Source of Funding: This research funded individually.
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Evaluation of Some Inflammatory Cytokines and Glycated Hemoglobin in Uncontrolled Type 2 Diabetes Mellitus with Nephropathy

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1Department of Clinical Laboratory Sciences, College of Pharmacy, University of Baghdad, Baghdad, Iraq, 2Ministry of Health / Medical City / National Center for Educational Laboratories, Baghdad, Iraq

Abstract

Background: Chronic hyperglycemia causes diabetic nephropathy (DN), which is a typical microvascular complication of type 2 diabetes mellitus. The pathogenesis of DN is not fully understanding. The inflammation may possess a significant role in the progression of DN in diabetic patients.

Method: The study accomplished at teaching laboratories of medical city, Baghdad, Iraq. It was included 50 uncontrolled diabetic type 2 patients with nephropathy, age range (40 – 78) years and 42 controlled diabetics type 2 without nephropathy, age range (35 – 52) years as a control group. The participants divided into two groups according to HbA1c measurement which is described as follows: < 7.5% of HbA1c describes controlled diabetes, and > 9% of HbA1c describes uncontrolled diabetes. The calculation of the Glomerular filtration rate (GFR) was used to determine the renal function for each participant. A venous blood sample was obtained to estimate HbA1c, fasting serum glucose (FSG), urea, creatinine, interleukin-6 (IL-6), interleukin-1 beta (IL-1β) and tumor necrosis factor – alpha (TNF-α).

Results: Significantly higher serum urea and creatinine levels in uncontrolled diabetic patients with nephropathy than controlled diabetic without nephropathy. The mean of HbA1c of DN group was higher as compare to diabetic control (DC) group. Results of IL-1β, IL-6 and TNF-α exhibits a significant raised level for diabetics with nephropathy, (p< 0.05) for IL-1β, (p< 0.05) for IL-6 and (p< 0.01) for TNF-α.

Conclusion: Raised serum levels of TNF-α, IL-1β and IL-6 in uncontrolled diabetic patients with nephropathy concurrently with raising the average HbA1c in them indicate that the inflammation may have a role in the advancement of DN in diabetic patients with poor glycemic control.

Keywords: inflammation, interleukins, type2 diabetes mellitus, HbA1c, hyperglycemia

Introduction

Diabetic nephropathy (DN) is an advanced renal disease designated by persistent albuminuria with reduction of glomerular filtration rate (GFR) due to uncontrolled hyperglycemia [1]. Metabolic changes occurred in diabetes causes deterioration of glomerular functions leading to DN that recognized by an accumulation of nitrogenous compounds like creatinine and urea with a consecutive deterioration in the GFR followed by arterial hypertension give raise to renal damage [2]. Immunological and inflammatory employ significant roles in DN and its progression, particularly IL-8, IL-6, IL-1β, and TNF-α, by modifying disease via various mechanisms. Regulation of inflammatory and immune responses by these cytokines through secondary pathways which are contributes to the pathophysiology of many diseases including diabetes mellitus (DM) [3]. Pathological alterations in DN are renal hypertrophy and hyperfiltration that are associated with inflammation processes which included release of proinflammatory and anti-inflammatory cytokines from many immune cells [4]. IL-1β is proinflammatory cytokines produced by macrophages and renal in inflammatory based diseases. It was involved in the declining of glomerular functions, leading to renal
damage in DN [5].

Interleukine-6 (IL-6) has both a pro-inflammatory cytokine and an anti-inflammatory effect in human, causes glomerular basement membrane thickening, enhances endothelial permeability and mesangial cell proliferation [6]. Tumor necrosis factor alpha (TNF-α), an inflammatory cytokine modifies glomerular functions and increased vascular endothelium permeability infiltration by inflammatory cells, grow extracellular matrix, generation of reactive oxygen species and blood flow interrupt are additional notable effects in renal structures [6]. The production of these proinflammatory cytokines, which triggered by the hyperglycemic status that happens in diabetic patients, followed in renal deterioration, either glomerulus or different structures [7].

Hyperglycemia or poor glycemic control was considered a good promotor of DN. Tightly controlled of blood glucose level is required to prevent or delay diabetic kidney disease [8]. Glycosylated hemoglobin (HbA1C) is biomarker of glycemic control in DM condition because it describes blood glucose levels in the last 60–90 days. Several studies determined that HbA1c were significantly correlated with cytokine concentrations in DN [9,10]. The purpose of this research to define the relation between glycated Hemoglobin and some inflammatory cytokines (IL-1β, IL-6 and TNF-α) in DN.

**Patients & Method**

The study accomplished at teaching laboratories of medical city, Baghdad, Iraq, a total 50 uncontrolled diabetics type 2 with stage 3-4 of nephropathy (UDN), age range (40 – 78) years and 42 controlled diabetics type 2 without nephropathy (DC), age range (35 – 52) years as a control group were enrolled in the study. The ethics committee approved the study of college of pharmacy, university of Baghdad, Baghdad, Iraq (No. UBCP-RECA 26102019). Exclusion criteria included: type 1 DM, gestational DM, multivitamin supplements, bleeding disorder, anemia, infections (e.g. hepatitis, malignant diseases). The glycemic file of the participants did evaluated by estimating HbA1c, FSG levels. The participants divided in to two groups according to HbA1c measurement which is described as follows: < 7.5% of HbA1c describes controlled diabetes, and > 9% of HbA1c describes uncontrolled diabetes [11]. Venous blood sample was collected to measure HbA1c using HPLC assay (Arkray, Germany) and fasting serum glucose (FSG) levels by enzymatic colorimetric method (Siemens USA). Serum urea nitrogen was measured by using urease/glutamate dehydrogenase coupled enzymatic technique (Dimension clinical chemistry System, DF21 Siemens, USA). Serum creatinine was measured by using modified kinetic Jaffe technique (Dimension clinical chemistry System, DF33B Siemens, USA). Enzyme linked immunosorbent assay (ELISA) technique was used for quantitative measurement of IL-1β, IL-6 and TNF-α by using human ELISA kit. Estimated Glomerular Filtration Rate (eGFR) was measured by modified diet renal disease (MDRD) equation for Creatinine and the equation adjusted for age and sex. [12], as shown in table (1). GFR measurement gives the basis for discovery and classification of chronic renal disorder. The eGFR of the UDN involved in the research classified as mild to moderate GFR reduction. The eGFR of the DC group classified as normal GFR.

<table>
<thead>
<tr>
<th>Stages</th>
<th>GFR(ml/min/1.73m2)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>≥ 90</td>
<td>Normal or elevated GFR</td>
</tr>
<tr>
<td>2</td>
<td>60-89</td>
<td>Mild GFR reduction</td>
</tr>
<tr>
<td>3</td>
<td>30-59</td>
<td>Moderate GFR reduction</td>
</tr>
<tr>
<td>4</td>
<td>15-29</td>
<td>Severe GFR reduction</td>
</tr>
<tr>
<td>5</td>
<td>&lt;15</td>
<td>Renal failure</td>
</tr>
</tbody>
</table>

**Statistical Analysis**

Statistical analysis was made employing SAS (Statistical Analysis System. Version 21). An Independent t-test sample was used to compare the serum levels of all parameters between UDN and DC groups. The variations between the groups were reflected to be significant at a p-value of ≤ 0.05.

**Results**

The laboratory and descriptive analysis for the participants were installed in table 2, which revealed the mean, standard deviation for these parameters and
p value. Calculations displays the mean of eGFR for patients group is 33.65, this value classified as moderate GFR reduction. The mean of eGFR for control group is 100.58 which classified as normal GFR values. Mean HbA1c of UDN group was 10.45±1.52% and that of DC group was 6.17±1.02%. The mean of HbA1c of UDN group was higher as compare to DC group, so the increase of HbA1c values associated with increased incidence of nephropathy in type 2 DM. Results of IL-1β, IL-6 and TNF-α displays a significant increased levels for UDN group, (p< 0.05) for IL-1β, (p< 0.05) for IL-6 and (p< 0.01) for TNF-α (table2).

<table>
<thead>
<tr>
<th>Table2: Laboratory and descriptive analysis for UDN and DC groups</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biochemical parameters</strong></td>
</tr>
<tr>
<td>Male /female ratio</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
</tr>
<tr>
<td>FSG (mg/dl)</td>
</tr>
<tr>
<td>Urea( mg/dl)</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73m2)</td>
</tr>
<tr>
<td>IL-1β (ng/ml)</td>
</tr>
<tr>
<td>IL-6 ( pg/ml)</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
</tr>
</tbody>
</table>

*p-value < 0.05 was analyzed significant and p-value<0.01 was analyzed highly significant

**Discussion**

Chronic hyperglycemia and inflammation are the major causative agent of DN[13]. Several metabolic, biochemical and hemodynamic changes act as a promoter to inflammation in DN. DN is a critical microvascular complications of type 2 diabetes mellitus that required early diagnosis and management to prevent irreversible renal failure[14]. Therefore, adequate control of blood glucose levels is needed to delay the progression of DN. Important markers were applied to determine the renal function which are blood urea and creatinine levels. The measurements of eGFR is depend on the amount of creatinine detected in a blood specimen. Raising serum creatinine levels result in increased GFR. Renal disorders are happened when eGFR is < 60ml / min / 1.73m2. Activation of the immune system and chronic inflammation are both implicated in pathogenesis of DN, researches have demonstrated that chemokines, cytokines, as well as immune cells as monocytes, lymphocytes, and macrophages are involved in pathogenesis and complications of type 2 diabetes mellitus[15]. In the current study, it noticed that IL-6 and IL-1β serum levels are significantly higher in UDN group than the levels mentioned in DC group as correspond with other studies[16,17]. IL-1 is recognized to be implicated in the deterioration of glomerular functions. It related to the secretion of prostaglandin by mesangial cells of kidney leading to changes in renal structure which are important in the development of DN[18]. In addition to this study, Sari MI et al. determined that serum IL-6 levels raised in patients with diabetic nephropathy [19]. Also a previous study has reported a significant difference in the IL-6 levels between a group of diabetic patients and a group of healthy people[20]. IL-6 also encourages growth and proliferation of mesangial cells. It has been recognized that increase proliferation...
and action of mesangial cells producing in extracellular matrix growth, glomerular basement membrane thickening, and glomerulosclerosis, eventually lead to DN [7]. Furthermore, IL-6 has non immune mediated mechanism that influence glucose metabolism by action on skeletal muscle cells, pancreatic islet cells and adipocytes [21,22]. Additionally, this research reveals that serum TNF-α levels in UDN group are significantly more than the serum TNF-α levels in DC group. Similarly, other studies show that increase risk of diabetic kidney disease associated with the increase levels of TNF-α in patients with type 2 DM [23,24]. TNF-α exert different effects to cells including apoptotic and necrotic cell death by direct and autocrine mechanism, also changes of endothelial permeability. TNF-α lead to impairment of glomerular capillary wall barrier function, hence increasing albuminuria [7].

In this study, the average of HbA1c in UDN group was significantly more than that of DC group, thus HbA1c may be used as an indicator of prognosis in the development of DM complications. Similar results were observed by other studies which found that increase HbA1c levels above 7.0% were significantly correlated with increased prevalence of nephropathy [25,26]. Past studies pronounced that age was crucial factor linked to renal function derangement. The old age and duration of diabetes were significant risk factors for nephropathy as an deal with this study [27,28]. Poor glycemic control shown by elevated mean HbA1c, and fasting serum glucose levels were significantly connected with increased predominance of nephropathy in this study.

**Conclusion**

Raised serum levels of IL-1b, IL-6 and TNF-α in uncontrolled diabetic patients with nephropathy concurrently with raising the average HbA1c in them indicate that the inflammation may have a significant role in the progression of DN. The occurrence and risk of DN increased with advanced age and poor glycemic control, so that the incidence and worsening of DN among type 2 diabetic patients could be limited or restricted by keeping tightly controlled HbA1c. The perception of the inflammatory response in DN is urged to identify novel anti-inflammatory plans for the intensive therapy to patients with DN.

**Conflict of interest:** There is no conflict of interest among the authors.

**Funding:** Self

**Ethical Clearance:** This study is ethically approved by the Institutional ethical Committee.

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The Impact of High Cortisol Level in Increasing Incidence of Infection Cases among Acute Stroke Patients

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Abstract

Background: Infection in the stroke was a treatment major problem, because it determines the prognosis. In the acute phase of stroke, high level cortisol may lead to a decreased immune system and patients tend to be more susceptible to infection. The correlation of serum cortisol level among acute stroke patients with incidence of infection was not fully investigated.

Objective: The aim of this study was to determine the relationship between initial serum cortisol levels and the incidence of infection in acute stroke patients.

Method: A prospective cohort study was conducted on 32 of acute stroke patients who admitted in RSUD Dr. Soetomo Surabaya during the period December 2015 to February 2016. Total of 2 cc to the venous blood within 48 hours in onset of stroke being examined of serum cortisol. During the 7 days of hospitalization, the patient was observed for signs of infection, both clinically and the investigation in accordance with the operational definition.

Results: In the infected group there were 8 people (50%) with high cortisol levels (p = 0.015; RR 15,000; 95% IK 1.583-143,171); 6 people were (50%) using urine catheter (p = 0.049; RR 6,667; 95% IK 1.067-30.085); And 6 people were (54.5%) using NGT (p = 0.035; RR 7,200; 95% IK 1.311 - 39,557). In multivariate analysis obtained that Odds Ratio (OR) 15,468 based on high cortisol and OR 7,469 based on NGT usage.

Conclusion: High cortisol levels and the use of NGT had the effect on the incidence of infection in acute stroke.

Keywords: Acute Stroke, Serum Cortisol Level, Infection Incidence

Introduction

Stroke was a major health problem in Indonesia. According to the National Basic Health Research data on 2013, the prevalence of stroke in Indonesia was 12.1 for each 1000 population, the number has revealed an increase in stroke prevalence when it was compared to the national data in 2007 which was 8.3 for each 1000 population. Based on Registry Stroke data 2014 infarct stroke was noted as the most frequent cases (67.1%), followed by bleeding stroke (32.9%) ².

In acute stroke, the systemic infection after the brain damage could be a symptom of the reduction competence of the immune system, that mediated by the central nervous system. The following period of stroke might be considered as a reaction to the stressful event. The red thread that links these two supersystems was the hypothalamus-pituitary-adrenal (HPA) axis ³. The main characteristics of the response to stress were the activation of the sympathetic nervous system and the HPA axis ⁴.

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There were several hypotheses that attempt to explain the effect of stroke on the HPA axis, one of them was the structural injury in the brain regions that play a role in the regulation of HPA axis, such as the brainstem, hypothalamus, pituitary, terminal lamina, Limbic hippocampus. Additionally, the lateralization of the nervous system also helps in explaining the several locations that affect the metabolic changes post stroke. In addition, recurrent stress might increase the adrenal sensitivity to ACTH, which turn to leads to the prolonged hyper cortisol. Cortisol was the responsible of primary hormone for the responses to the stress. In the event of exposure to stress the increased of circulating cortisol were inhibits the HPA activity at the hypothalamus and pituitary levels.

Increased cortisol suggests a severe inflammatory process that will lead to increased temperature, fibrinogen, leukocyte count, β thromboglobulin and IL-6[11]. Cortisol levels remain high within 7 days post-acute stroke and return to the normal limits approximately after 3 months. The highest cortisol levels were estimated to occur in the first 48 hours marked by the highest deactivation of monocytes.

Whereas, In acute stress conditions, cortisol also facilitates the release of anti-inflammatory mediators such as IL-4 and IL-10 that have apoptotic effects on immune cells[13]. However, in chronic exposure, high levels of cortisol prevent this effect. The effect of cortisol on these cytokine signals was likely to optimize the occurrence of inflammation. Complications of infection in stroke was a major problem in the treatment of stroke patients. Reported that 5-65% of acute stroke patients with the most common types of infection were urinary tract infections (16%) and pneumonia (12%). Factors that caused the Incidence of infection in high stroke includes: (1) frequent invasive medical procedures such as surgery, catheterization, mechanical ventilation, nasogastric tubing, (2) bed rest, (3) exposure to various drug-resistant bacteria, and (4) the swallowing disorders resulting from the lesions in the central nervous system and causing aspiration. However, the correlation of serum cortisol level among acute stroke patient with incidence of infection was remain unclear.

**Materials and Method**

This study was prospective cohort with analytic observational design. Affordable populations as the sample of this study were an acute stroke patients who admitted in Department of Neurology, Dr. Soetomo hospital, Surabaya, Indoensia during the period December 2015 to February 2016. The subjects were divided into two groups, namely the subject group of acute stroke patients with high cortisol levels and acute stroke subjects group with normal cortisol levels. Of total 32 samples, 16 samples were included in the high cortisol group and 16 were included in the normal cortisol group. In this study, several variables that measured were serum cortisol levels, the incidence of infection, age, body temperature, use of urine catheter, use of nasogastric tube (NGT).

Serum cortisol level are measured by blood samples collection as much as 3 ml from the study subjects within the first 48 hours of stroke onset, at 6-8 am then being examined in laboratory. High serum cortisol levels, when the results obtained on the examination more than 22.40 μg / dL. Normal cortisol serum levels, when the results obtained on the examination between 4.30 - 22.40 μg / dL.

Statistical analysis was performed with SPSS version 22.0. The collected data was analyzed by Chi Square test for categorical data and unpaired t test for normal distributed numerical data. Differences in the proportion of infections in both groups were tested using Chi Square test.

**Results**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Infection (+)</th>
<th>Infection (-)</th>
<th>Total</th>
<th>p</th>
<th>RR (IK 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>6 (54,5%)</td>
<td>5 (45,5%)</td>
<td>11 (100%)</td>
<td>0.035</td>
<td>7,200</td>
</tr>
<tr>
<td>No</td>
<td>3 (14,3%)</td>
<td>18 (85,7%)</td>
<td>26 (100%)</td>
<td></td>
<td>(1,311-39,557)</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>23</td>
<td>32</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In the group of subjects infection, 6 (54.5%) was got NGT and 3 people (14.3%) did not use NGT. The chi square test showed statistically significant difference of proportion with $p = 0.035$ and relative risk (RR) was 7,200 (95% CI 1,311 - 39,557) (Table 3). This means that subjects with the use of NGT had a relative risk for infection 7.2 times compared to the subjects without NGT use.

**Table 2. Correlation between cortisol rate and infection incident**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Infection (+)</th>
<th>Infection (-)</th>
<th>Total</th>
<th>$p$</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol Rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>8 (50%)</td>
<td>8 (50%)</td>
<td>16</td>
<td>0.015</td>
<td>15,000</td>
</tr>
<tr>
<td>Normal</td>
<td>1 (6.25%)</td>
<td>15 (93.75%)</td>
<td>16</td>
<td></td>
<td>(1,583-143,171)</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>23</td>
<td>32</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In the group of subjects infection, 8 (50%) had high cortisol and 1 person (6.25%) with normal cortisol levels. By using chi square test statistically significant difference with $p = 0.015$ and Relative Risk equal to 15,000 (95% CI 1,583 - 143,171). This means that subjects with high cortisol levels had a relative risk for infection of 15,000 times compared to the subjects with normal cortisol levels (Table 3).

**Table 3. Analysis result of multivariate logistic regression**

<table>
<thead>
<tr>
<th>Coefisien</th>
<th>SE</th>
<th>Wald</th>
<th>Df</th>
<th>P Value</th>
<th>OR</th>
<th>IK 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Min</td>
</tr>
<tr>
<td>Cortisol rate</td>
<td>2.739</td>
<td>1.232</td>
<td>4.941</td>
<td>1</td>
<td>0.026</td>
<td>15,468</td>
</tr>
<tr>
<td>NGT</td>
<td>2.011</td>
<td>1.020</td>
<td>3.887</td>
<td>1</td>
<td>0.049</td>
<td>7,469</td>
</tr>
<tr>
<td>Constanta</td>
<td>-3.597</td>
<td>1.254</td>
<td>8.222</td>
<td>1</td>
<td>0.004</td>
<td>0.027</td>
</tr>
</tbody>
</table>

Based on this multivariate analysis (table 4), it revealed that the variables affecting the incidence of infection cortisol levels and the NGT use with the greatest relation strength was cortisol content with OR = 15,468.

**Discussion**

In this study, we found 20 subjects with infarct stroke and 12 subjects with intracerebral hemorrhage stroke. There was no significant difference of cortisol
content based on the stroke type with p = 0.716. This was consistent according to the results of a meta-analysis of various studies that found elevated cortisol levels were more associated with morbidity, mortality and stroke severity, compared to the stroke types. In this study, we obtained acute stroke patients with high cortisol levels of 16 subjects or 50% of the total subject. In the patients with acute stroke, there was an increase or abnormality of ACTH secretion and cortisol associated with extensive infarction, poor functional outcome and increased mortality.

In this study, 8 subjects with diabetes mellitus and 24 subjects did not suffer from the diabetes mellitus. There was no significant difference in the proportion of diabetes mellitus status between the two groups in this study (p = 0.220). Chiodini in his study also mentioned that the increase in cortisol levels was more related to the complications of diabetes mellitus and it was the degree of complication. Meanwhile in this study was not studied about the complications and degrees of complications in diabetes mellitus that occurred in the study subjects.

Glucocorticoids affect vascular tone by increasing vascular resistance and causing secondary hypertension. However, there was no significant difference in proportion between the two groups in our study (p = 0.5654). This might be due to our study, that the hypertension was thought to be primary hypertension that was more associated with vascular risk factors and not secondary to the high levels of glucocorticoids.

Urinary catheter insertion action could bring the organisms from the urethra directly into the bladder. Most strains of bacteria entering through the urinary catheter could multiply rapidly within a day and persist for up to weeks causing urinary tract infection (UTI). This was consistent with our results where in the group with infection there were 6 subjects (50%) using catheters and 3 subjects (15%) did not. From the analysis with chi square test, there was a statistically significant difference of proportion with p = 0.035 and relative risk was 7.200 (95% IK 1.311-39.557). Based on these data it could be said there was a significant relationship between the use of NGT with the incidence of infection, where subjects who use NGT have a risk of 7.2 times to experience the incidence of infection than subjects who did not use NGT. The use of NGT in this study was a significant confounding factor for the occurrence of infection.

Chen et al mention that the brain infarction could lead to the systemic immunodepression which was predisposes to the infection. Stroke induces widespread apoptosis of lymphocytes and a shift in the production of Th1 to Th2 with immune-system depression. Choi et al mentioned in his research that cortisol exposure was known to reduce the telomerase activity in human T lymphocytes. Meanwhile, Mavoungou et al found that cortisol decreased the ability of NK cells to kill target cells. Cortisol inhibits the expression of cytotoxic receptors from NK cells Transcription level of the regulating gene.

Although several studies have examined the relationship between the post-stroke and the incidence of infection, there have been no specific studies that been examining the association between serum cortisol levels and the incidence of infection in acute stroke patients. Therefore, this study was the first study to find the relationship between serum cortisol levels and the incidence of infection in patients with acute stroke. The limitations in this study were the presence of the other confounding factors that could not be controlled, such as the use of urine catheter and NGT. The advantages of this research were to use a prospective cohort research design which was the best observational research design to find the cause and the effect relationship.

Conclusions

In conclusion, this study revealed that high serum cortisol levels and the use of NGT among stroke patients
may contributed to the incidence of infection.

**Ethical Clearance:** This research process involves participants in the survey using a questionnaire that was accordant with the ethical research principle based on the regulation of research ethic committee. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, nonmaleficence, and justice.

**Conflict of Interest:** The author reports no conflict of interest of this work.

**Source of Funding:** This study is done with individual funding.

**References**


Time Recommendation and Alternative Parameters for Severe Maxillofacial Trauma Reconstruction

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Abstracts

Background: Severe maxillofacial trauma often associated with other injuries, therefore the delay of reconstruction often occur until the patients are stable. Early reconstruction results a better facial function and appearances.

Aim: The aim of this study to give the recommendation and alternative parameters for severe maxillofacial trauma reconstruction.

Methods: The method of this study is adult patients with bimaxillary and bilateral maxillofacial trauma were eligible for this study, while pregnant women were excluded. Nine patients with maxillofacial trauma were involved in this study and we measured 15 facial anthropometric and 41 lateral cephalometricon 7, 14, 21 days compared to 3 months post-reconstruction.

Results: The result of this study showed reconstruction can be planned 14 days after trauma for lower jaw fracture and 21 days after bimaxillary fracture. Lateral cephalometry was a reliable method to measure facial edema following surgery which combined facial anthropometry with lateral cephalometry using parameters that are not affected by edema. This can be applied as an additional guiding tool in surgical planning for maxillofacial trauma patients especially those with bimaxillary and bilateral fractures.

Conclusion: Combination of anthropometric and cephalometric parameters which are not affected by edema can be applied as an additional guiding tool in surgical planning for maxillofacial trauma patients.

Keywords: Facial Anthropometric, Lateral Cephalometry, Facial Edema, Severe Maxillofacial Trauma.

Introduction

Maxillofacial trauma patients, especially with panfacial fracture, often requires of following surgery that associated with the facial deformity which is often indicated repairing natural architecture of facial bone and leaving minimal traces as possible¹. In developing countries, almost 50-70% of traffic accidents remain the leading cause of facial trauma¹.

Anthropometry is the measurement of living subjects². Head and facial anthropometry measurements can be used together with cephalometry, Computed Tomography (CT), and Magnetic Resonance (MR) in preparation for a patient undergoing plastic and reconstructive surgery³. The lateral cephalometry may help evaluate the loss of vertical dimension and skeletal relations between the arches. Easy to access the equipment and low cost could provide a correct surgical planning when analysed appropriately⁴.

Modified techniques using anthropometric combined with cephalometric examination are easy and inexpensive. Facial edema can be assessed using cephalometric measurements, Moreover a combination between anthropometric and cephalometric for surgical planning could be used as an alternative for surgeons. Therefore, we aimed to assess the time recommendation and alternative parameters for severe maxillofacial trauma reconstruction⁵.

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Method

This research uses experimental study design, posted test only group design. In other side, this study used adult patients with bimaxillary and bilateral maxillofacial trauma were eligible for this study, while pregnant women were excluded. After obtaining the informed consent of the patients underwent surgery. All of them were operated following the same procedure in the same hospital by three different surgeons at the same level of experience. Facial anthropometric and lateral cephalometric measurements were taken ON day 7, 14, 21 and 3rd month postoperatively. Facial anthropometry was measured using a standardized anthropometric tool such as spreading and sliding caliper, its showed on Figure 1 and lateral cephalometry using Vistadent software (GAC Techno Center, Birmingham, United Kingdom) on Figure 2.

Figure 1. Anthropometric measurement using spreading caliper and sliding

Figure 2. Cephalometric analysis using vistadent software, the dots which are refer to landmarks on cephalometry are being filled, and computer will calculate it.

Figure above it’s the figure that used by researcher in this study. In other side, researcher used some data. First, data from 15 facial anthropometric and 41 lateral cephalometric which measured on day 7, 14, 21 were compared to those taken within 3 months after post-operatively. It was to assess whether facial anthropometric and lateral cephalometric measurements following the surgery were affected by edema. Second, the measurements of lateral cephalometric on day 7, 14, 21 were compared to third (3) month post-operatively to assess the time resolution of facial edema after the operation. The paired samples T-test was used to compare anthropometric and cephalometric variables on day 7, 14, 21 with third (3) month postoperatively. Data were analyzed using SPSS version 11.0 for windows.

Result

Between September 2012 and March 2013, the data were collected from nine patients consisted of seven males and two females between 17 and 31 years old (median 24 years). Seven out fifteen (7/15) anthropometric parameters (zy-zy, go-go, ex-ex, sn-c, go-cdl1, go-cdl2, gn-go1) were affected by edema (p <0,05), while the remaining eight parameters remained constant throughout day 7, 14, 21 and 3rd month postoperatively. All cephalometric parameters remained constant throughout day 7, 14, 21 and 3rd month postoperatively except 4 parameters (Ls-NsPog’, A’-SS; Ls1u-Ls; Pog-Pog’). The cephalometric parameters are able to assess the time resolution of facial edema, such as basic upper lip thickness (A’-SS), thickness of vermilion of the upper lip (Ls1u-Ls), and soft tissue thickness of chin (Pog-Pog’). Basic upper lip thickness (A’-SS) and soft tissue thickness of chin (Pog-Pog’) on day 7 were significantly different compared to 3rd month postoperatively (p <0,05) while basic upper lip thickness on days 14 was significantly different compared to 3 months postoperatively (p <0,05). There was no further reduction of facial edema in the lower jaw after day 14 and in both upper and lower jaw after day 21.

This study showed that the edema of the lower jaw disappeared within two weeks after the reconstruction of maxillofacial trauma and edema of the face within three weeks. The faster resolution of edema on the lower jaw could be explained by the presence of the largest space in the fourth layer of the face, the premasseteric space, allowing greater movement leading to greater of edema reduction6 (Figure 3).

Figure 3. Soft tissue spaces of the face: upper temporal, prezygomatic, masticator and premasseter space. Adapted from Mendelson, B. ‘Facelift anatomy,
On the figure 3, we considered the surgery as the moment of trauma because the surgical trauma itself is proportional to the actual trauma that causes the fractures. The morphology of the face returned close to 90% of the baseline facial scan at 3rd month while, scanning after sixth months was used as the baseline. In our study, facial morphology returned to the baseline at week 3, while the scans recorded on the third month was used as the baseline.

The fracture of facial bone remodeling process begins in responding to an interrupted blood supply. At the end of the first week after trauma, non-perfused bone is substituted by a new bone that could be visible 2 to 3 weeks after trauma. Facial bone remodeling is faster than in long bones, because of the outstanding circulations allows faster recovery. Normally, facial bone healing takes around 4 to 6 weeks in midface region and 10 to 12 weeks in mandible region. Callus could be seen at 2 to 3 weeks after trauma, while performing reconstruction after 4-6 weeks in the midface and after 10-12 weeks in mandible probably will need osteotomy because of the bone is reunion.

The optimal results of surgery for repail of panfacial fracture can be obtained two weeks after the trauma. In some circumstances, even early surgical intervention should be avoided, such as cervical spine injury. Primary repair can be successfully completed up to 3 weeks after injury. The results of this study indicate that reconstructive surgery should be planned 14 days after trauma for lower jaw fracture and 21 days after bimaxillary fracture. The surgeons have an alternative option to immediately operate on a maxillofacial trauma patient with subsequent surgery risk if postoperative deformity occurs or wait until facial edema resolves.

Eight anthropometric parameters were not affected by edema, they remain constant for postreconstruction. This can be explained by the presence of facial ligaments cause the area to become more fixed, therefore, it is more resistant to edema (Figure 4).
Figure 4 showed that forty-one cephalometric parameters were not affected by edema. We also found that upper and lower facial height (tr-gn, n-gn, UFH, LFH) were not affected by edema. We suggest these parameters can be used when edema appears on the face.

From this study I can be showed that maxillofacial trauma caused by severe ballistic or avulsion injuries. Some of that injuries can be creates complex composite defects for the human13.

**Conclusion**

Swelling due to edema of the lower jaw disappeared within two weeks after the reconstruction while, swelling of the face disappeared within three weeks for maxillofacial trauma. Reconstruction can be planned 14 days after trauma for lower jaw fracture and 21 days after bimaxillary fracture. Lateral cephalometry was a reliable method to measure facial edema following surgery which combined facial anthropometry with lateral cephalometry using parameters that are not affected by edema. This can be applied as an additional guiding tool in surgical planning for maxillofacial trauma patients especially those with bimaxillary and bilateral fractures. This approach has the advantage of accuracy, convenience and inexpensive than other scan-based techniques.

**Conflict of Interest:** Nil

**Source of Funding:** Self

**Ethical Clearance:** This study was approved by Ethical Commission of Health Research Faculty of Medicine University of Airlangga.

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Development of Geogebra Learning Media Based on Statistical Reasoning on Statistics Materials of Junior High School Students and its Influence for the Inteligent of Student

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Abstract

This research aimed to develop geogebra learning media based on statistical reasoning. For the purpose of this research and development, ADDIE model by Dick & Carry was used. This research and development produced geogebra learning media based on statistical reasoning for junior high school students. Class environment that applies technology based on statistical reasoning is another variable besides genetic which is adaptive. The integration of new technologies for education is no longer an alternative; it has become an obligation and even guidance to develop these knowledge-sided. Through geogebra, students are directed to build their knowledge not just to memorize rules. The class that applies technology based on this statistical reasoning will develop students’ intelligence.

Keywords— geogebra, statistics, statistical reasoning, intelligence

Introduction

The rapid development of data in the era of big data like now makes the need for statistics not only needed in the field of mathematics (1), (2), (3), (4), (5). Given the importance of statistical reasoning, several researches related to statistics refer to statistical reasoning (6). Statistical reasoning must be output in statistical learning (7). In addition, statistical reasoning is also an input in statistical learning as a consideration of the process in statistical learning (8).

However, current statistical reasoning research is more focused on the level of statistical reasoning. Research related to learning has not had a significant impact. There are no specific learning techniques or methods to improve statistical reasoning abilities (9). It is explained further that there is no significant difference between the ability of students who are taught by using traditional learning and student-centered statistical learning (10). There are important components considered in statistical reasoning besides statistical learning methods, including the use of technology, curriculum, and assessment (11).

The integration of new technologies for education is no longer an alternative; it has become an obligation and even guidance to develop these knowledge-sided (12). One of the six principles of learning design is a technological device (13), (14). It was important for many institutions to make vast investments in such innovations with the intention of incorporating digital computer tools into their educational curricula (15),(16).

Learning media are various kinds of tools and equipment that can be used to improve or also complement the efforts of teachers in ensuring interesting learning for students (17). In learning statistical reasoning, technology-based learning media are needed. Integrating the use of appropriate technology can facilitate students to test their expectations, explore, and analyze data interactively (18).

But in reality, the use of technology for learning is still rarely done. Innovative strategy were not appear in teaching (15). Integration of information technology in reasoning is still rare (7),(8). The use of media in statistics is usually only to produce statistical measurements or to draw graphs. This is done in order to encourage educational efforts aimed at assimilating competencies
from the use of digital technology (19). One of the technology learning media that is currently being developed is GeoGebra.

GeoGebra is software that can be one of the best choices for learning media that has many benefits for geometry, algebra, and statistics to be more easily understood (20). GeoGebra empowers pupils to discover, detect patterns, make a assume, illustrate, organize data (21). The majority of current research is on the implementation of GeoGebra in learning geometry and algebra, statistical learning using GeoGebra is still very minimal to be studied. GeoGebra offers geometry, algebra and calculus features in a fully connected, compacted and easy-to-use software environment (22). In statistical learning, technology-based learning media are used not only to produce statistical measures, draw graphics, or analyze data but also to help students visualize concepts and develop the understanding of abstract ideas through simulations. It is expected that by using GeoGebra learning media, students’ statistical reasoning can be improved. The indicator of reasoning ability is to use or interpret statistical models such as formulas, graphs, tables to draw conclusions; solve problems according to the method; communicate information effectively visually, numerically and verbally; assess the accuracy of conclusions based on the quantity of information (23).

Based on the urgent need for technology-based learning media, the researchers are interested in developing GeoGebra learning media based on statistical reasoning.

**Research Methodology**

This research used development research with ADDIE development models by Dick & Carry. Some phases of the implementation of research and development of ADDIE (Analysis (A), Design (D), Development (D), Implementation and Evaluation (I&E)) model are as follows (24):

**Analysis Phase**

This phase includes analysis activities: 1) needs based on the unavailability of statistical learning media that support the implementation of learning in the classroom, where only teachers and students books are available; 2) literature, obtained from the results of studying student books and teachers books that the objective of statistical learning existing; 3) analysis of learning in the classroom obtained results that in learning, the teacher only uses technology learning media namely LCD to display ppt and students books to students.

**Design Phase**

At this phase, research planning was carried out which includes:

1) Exploring Potential and Problems

The problem that arises in the schools studied was the use of new mathematics learning media limited to the use of power points to present material and the lack of students’ abilities in statistical reasoning;

2) Literature Study and Information Collection

The competencies expected to arise in junior high school students learning statistics are related to statistical reasoning. Statistical reasoning is the way people make excuses with statistical ideas and make “sense” of statistical information which includes skills in making interpretations based on a collection of data, making statistical summaries related to data, where students need to combine ideas about data, make conclusions, and interpret results statistically (24).

**Product Development Phase**

1) Validation

At this phase, a GeoGebra learning media expert was validated by 4 validators, 2 from GeoGebra media experts and 2 material experts. The validation data were then analyzed using the following formula:

\[ P_m = \frac{\sum x_i}{32} \times 100\% \]

Note:

\[ P_m = \text{percentage of eligibility for all items} \]

\[ \sum x_i = \text{total score obtained for all valid items by validators} \]

If the value of \( P_m \geq 74\% \), the media is a valid medium, and therefore it does not need to be validated.

2) Revision

After validation, a design revision was conducted based on validators’ input. This input was used as a basis for improving learning media.
1.1 Implementation and Evaluation Phase

The initial product was then tested on a limited basis. After being tested, students were asked to provide responses related to the learning media used. The students’ response data were then analyzed using the following formula:

\[ P_a = \frac{\sum x_i}{N \sum y_i} \times 100\% \]

Note:

- \( P_a \) = percentage of eligibility for all items
- \( \sum x_i \) = total score obtained for all items by the user
- \( \sum y_i \) = the number of expected scores for all items by the user
- \( N \) = the number of students who filled out responses

If the value of \( P_a \geq 74\% \), the media is said to be practical, so there is no need to revise it.

The results of students’ responses were then used to revise product learning media 1. The results of this revision are referred to as the final product whose results are given back to students. This is done to test whether the learning media has been effective for improving students’ reasoning or not. Data analysis of the effectiveness of this media was obtained from the results of the pre-test and post-test of students’ statistical reasoning abilities. The mean values of pre-test and post-test scores were compared using paired sample t-tests on the condition that the sample must be normally distributed.

### Result and Discussion

#### Initial Product Development Results

At this phase, geogebra media was developed in junior high school statistics material. The preparation of the media was done based on the competencies that exist in the applicable curriculum.

#### Experts’ Validation Results

The results of the learning media validation are presented in Table 1 below:

**Table 1. Results of Learning Media Validation**

<table>
<thead>
<tr>
<th>Validator</th>
<th>Percentage</th>
<th>Caption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media 1 expert</td>
<td>84.37%</td>
<td>Valid</td>
</tr>
<tr>
<td>Media 2 expert</td>
<td>90.62%</td>
<td>very valid</td>
</tr>
<tr>
<td>Material 1 expert</td>
<td>90.62%</td>
<td>very valid</td>
</tr>
<tr>
<td>Material 2 expert</td>
<td>87.50%</td>
<td>very valid</td>
</tr>
</tbody>
</table>

From the results of the validation above, there was a value that represents that the media were valid and very valid. Input given by the media expert validators is that learning media needs revision, namely the addition of sliders to facilitate changes in the amount of data, refinement of layout, changes in writing, and hiding features that are not needed.

#### Trial Results

The next step after the media has been validated and then revised is a trial of 1 product. The test was conducted at a junior high school with 30 students. Students were given statistical learning by utilizing the learning media that had been developed. After learning, students were given students’ response questionnaire to be filled in. The students’ response data were then analyzed using formula \( P_a \) as stated in section 2.4 above. From this questionnaire, it was ascertained that learning media was very practical to use. It can be seen from the analysis of students’ responses that have a value \( P_a \) of 87.7%. The results of this study are in accordance with the function of the media, one of which is to guarantee interesting learning for students (17), their interest improved greatly and teaching efficiency boosted significantly (19). From the students’ response questionnaire, there were suggestions/inputs from students that it is necessary to get used of using the geogebra application to make it easier. This input is used for further learning.

#### Effectiveness Test Results

The effectiveness data were obtained from the scores of pre-test and post-test results of trial 2. Data were obtained from questions related to statistical reasoning. Paired t-test data analysis was performed with the help of SPSS with a significance value of 0.01. The SPSS test results showed that the pre-test and post-test score were normally distributed. It was also obtained data as follows:
Table 2. Paired Samples Test

<table>
<thead>
<tr>
<th>Paired Differences</th>
<th>t</th>
<th>df</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>Std. Deviation</td>
<td>Std. Error Mean</td>
<td>99% Confidence Interval of the Difference</td>
</tr>
<tr>
<td>Pretest - Posttest</td>
<td>-1.79000E1</td>
<td>7.18403</td>
<td>1.31162</td>
</tr>
</tbody>
</table>

From the above data, it was obtained that $t_{\text{count}} = -13.647$. This value was then compared with the table value with $\alpha = 0.01$ and degrees of freedom $= 29$ that is equal to -2.462. Because the value of $t_{\text{count}} < t_{\text{table}}$, $H_0$ was rejected, which means the average score of post-test is bigger than the pre-test value. It can be concluded that the developed geogebra learning media met the effectiveness criteria which could improve students’ statistical reasoning abilities. This also affects the intelligence of students. One of intelligent components is reasoning. Some experts even explain that reasoning and intelligence refer to the same thing (25), (26).

The factors that influence intelligence are not only biological aspects, namely genetic. Environmental factors will also greatly affect the development of human intelligence (27), (28). Intelligence is not entirely due to biological variables. Intelligence is also influenced by other variables. Any variables can affect intelligence if this variable is related to adaptive nature (29). Class environment that applies technology based on statistical reasoning is another variable besides genetic which is adaptive. The class that applies technology based on this statistical reasoning will develop students’ intelligence.

Geogebra is software for developing mathematical ability, with the use of geogebra, students can manipulate various types of mathematical constructions from the simplest to the most complex. The impact is students will understand better and will open the way for further learning (30). It is emphasized again that geogebra can facilitate students in exploring, representing, and analyzing one concept with another concept (23). As explained in the theory that technology-based learning media is not only used to calculate statistical measures, draw graphs, and also analyze data but it can be used to help students visualize concepts and develop understanding statistics abstract ideas.

**Final Results**

The final result of this development research is the learning media of geogebra software on grade VIII SMP/ MTs statistical material. The resulting file is a ggb file format. To operate, it is necessary to install geogebra software on PC / laptop used, or by visiting the geogebra tube website so that it can operate without installing geogebra software.

**Conclusion**

From the process and results of the research stated above, it can be concluded that the geogebra learning media developed is effective which can improve students’ statistical reasoning abilities and intelligence.

**Acknowledgment:** This study supported by Universitas Muhammadiyah Ponorogo as a funder.

**Ethical Clearence:** Yes.

**Conflict of Interest:** No

**Source of Funding:** Authors

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The Association between Blood Glucose Control Measured with Serum HbA1c Level with Peroneal Motor Nerve Conduction Velocity in Patients with Type 2 Diabetes Mellitus with Polyneuropathy

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Abstract

Background and Objective. The poor control of blood glucose levels in patients with diabetes mellitus is a major risk factor from various complications of diabetes mellitus, such as polyneuropathy. One of the nerves that are often affected in the case of diabetic neuropathy is the peroneal nerve. This study aimed to determine the association between blood glucose level control measured with serum hemoglobin A1c (HBA1c) levels with peroneal motor nerve conduction velocity in type 2 diabetes mellitus patients with polyneuropathy.

Methods. The study was conducted on diabetic neuropathy patients who fulfilled inclusion and exclusion criteria in neurology unit and diabetes unit Dr. Soetomo General Hospital Surabaya on a consecutive basis from February to July 2013. The serum HbA1c level and nerve conduction velocity in peroneal motor nerve were examined; then, the results were grouped into two groups which were serum HbA1c <7% and ≥7% and NCV in normal and low peroneal motor nerve

Results. There were 26 subjects, consisting of 6 subjects with serum HbA1c <7% and 20 subjects with serum HbA1c ≥ 7%. There was an association between blood glucose level control measured by serum HbA1c levels and NCV in peroneal motor nerve with statistical significance of p = 0.0018 (Odd ratio 15; 95% IK 1.397 - 161.045).

Conclusion. There was an association between blood glucose level control measured by serum HbA1c levels with NCV in peroneal motor nerve in type 2 diabetes mellitus patients with polyneuropathy.

Keywords: polyneuropathy, HbA1c, nerve conduction velocity, diabetes mellitus

Introduction

Diabetes Mellitus (DM) is a group of metabolic disorders characterized by hyperglycemia, due to damage to insulin secretion, insulin work, or both. Factors that contribute to hyperglycemia include reduced insulin secretion, decreased glucose use, and increased glucose production, and the etiology of diabetes mellitus. The number of patients with DM in the world has increased. Based on the Central Bureau of Statistics’ data in Indonesia in 2003, from Indonesia’s population above 20 years old of 133 million people, the number of DM patients in urban areas is 8.2 million and 5.5 million in rural areas. Based on the pattern of population growth, it is estimated that by 2030 the population over the age of 20 is 194 million; thus, it is estimated that there are 12 million people with diabetes mellitus in urban areas and 8.1 million in rural areas. Based on the observations in Surabaya (reported in 1993), the prevalence of symptomatic diabetic neuropathy was high (51.4%), and it will increase with the duration and severity of diabetes.
Chronic hyperglycemia in diabetes is associated with damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Poor control of glucose levels is a major risk factor for complications of diabetes mellitus. The incidence of diabetic neuropathy is related to male gender, irregular blood sugar control and hypertension in diabetes patients. The American Diabetes Association (ADA) recommends the A1c target of less than 7%, while the American Association of Clinical Endocrinology recommends less than 6.5%. Efficient glucose control and monitoring using HbA1c can reduce complication of diabetes efficiently.

The pathogenesis of diabetic neuropathy involves many factors such as metabolic, vascular, oxidative stress, and neurohormonal growth factor deficiency. Diabetic neuropathy results from complex interactions between metabolic factors directly related to hyperglycemia and structural changes such as degeneration of axonal and demyelination caused by microangiopathy. This mechanism can involve a nerve called mononeuropathy or multiple called multifocal neuropathy. Both acute and chronic damage to peripheral nerve will result in anatomical and physiological changes in the peripheral nerve and will damage the structure consisting of myelin, axons, and buffer layers.

In the case of polyneuropathy, the longest nerve fibers have the highest risk of exposure, while the short fibers are less risky. Polyneuropathy is a length-dependent neuropathy, because the longest nerve fibers in the body are those that travel from the lower back to the legs; thus, in case of polineuropathy, the initial weakness or sensory impairment is the leg. The peroneal nerve in the limb contains motor and sensory fibers; thus, in the neuropathy of the peroneal nerve, the patient may experience both sensory weakness and impairment. This study aimed to determine the association between blood glucose level control measured with serum HbA1c levels with NCV in peroneal motor nerve in patients with type 2 diabetes mellitus with polyneuropathy.

Method

This research was a cross sectional study by using case-by-case sampling method (sampling from consecutive admission) until the samples were collected. The study was conducted in neurology unit, diabetes unit, and electromyography room of Dr. Soetomo General Hospital Surabaya on February - July 2013. The subjects were selected according to the inclusion criteria including patients with type 2 diabetes mellitus who had polyneuropathy (Toronto Score ≥ 5), aged 45-70 years old and willing to participate in the research. The exclusion criteria were anemia, history of alcohol addiction, had impaired renal function, impaired liver function, complication of diabetic ulcers in the legs which complicated the examination of NCV (Nerve Conduction Velocity) in peroneal motor nerve.

The variables of this study included independent and dependent variables. The independent variable is blood glucose level control measured by serum HbA1c levels. Meanwhile, the dependant variables was NCV in peroneal motor nerve.

The research material was a blood sample of the veins taken when the patient visited to the electromyography room in Dr. Soetomo General Hospital Surabaya before the neurophysiology examination. The research instrument used a patient data collection sheet. Examination of NCV in peroneal motor nerve was conducted with EMG tool in electromyography room of Dr. Soetomo General Hospital Surabaya with Caldwell brand and interpreted by EMG consultant neurologist. Serum HbA1c examination was performed in laboratory that has been certified internationally for HbA1c examination which was in Prodia clinic laboratory, Surabaya.

Patients with type 2 diabetic polyneuropathy were screened based on the inclusion and exclusion criteria to obtain the research samples. The samples were then examined for serum HbA1c levels and NCV in peroneal motor nerve. After obtaining the data, it was performed a statistical test to determine the association between blood glucose level control measured with serum HbA1c levels with NCV in peroneal motor nerve in patients with type 2 diabetes mellitus with polyneuropathy. The data were analyzed using a software (SPSS 17) to find out whether there was an association between two variables by using chi square test.

In this study, the normal value of NCV in peroneal motor nerve was >40 mm/sec according to the standards applied in the Electromyography room Dr. Soetomo General Hospital Surabaya by using the Caldwell tool. The analysis was divided into two groups: NCV in peroneal motor nerve >40 mm/sec (normal) and ≤40 mm/sec (slowing). Each patient in this study obtained two results of NCV in peroneal motor nerve.
the right and left side. For analysis purposes, if both sides obtained a normal result, it was considered normal. Meanwhile, if both sides or one side were slower, it was considered to experience a slowdown.  

Result

The overall subjects of the study were 26 subjects consisting of 9 males (34.6%) and 17 females (65.4%), whereas in the HbA1c ≥7% group, 7 (77.8%) males and 13 females (61.5%) (table 1). The gender difference in each group was not statistically significant (p = 0.668). Mean age of DM patients with polyneuropathy with HbA1c <7% was 56.67 ± 6.861 years old while DM patients with polyneuropathy with HbA1c ≥7% was 56.90 ± 5.486 years old (table 1). The mean age difference in each group was not statistically significant (p = 0.693).

From DM patients with polyneuropathy with HbA1c ≥7%, 9 (69.2%) were suffering from DM <10 years old and 11 subjects (84.6%) suffered from DM ≥ 10 years (table 1). The difference in percentage of long-suffering DM was not statistically significant (p = 0.645). From DM patients with polyneuropathy with HbA1c ≥7%, 11 (73.3%) had normal serum TG level and 9 (81.8%) had high serum TG level (Table 1). The percentage difference in serum TG level was not statistically significant (p = 1.000).

In low NCV in peroneal motor nerve group, 6 subjects (66.7%) were males and 10 subjects (58.8%) were females (table 2). Different percentage of gender in each group was not statistically significant (p = 0.668). The association between long-suffering DM and NCV in peroneal motor nerve was shown in Table 2. In DM subjects with polyneuropathy with low NCV in peroneal motor nerve, 8 subjects (61.5%) suffered from long-standing DM <10 years and 8 subjects(61.5%) suffered DM ≥10 years old. The difference in percentage of long-suffering DM in both groups was not statistically significant (p = 1.000).

The association between the serum triglyceride (TG) level and NCV in peroneal motor nerve was shown in Table 2. In DM patients with polyneuropathy with low NCV in peroneal motor nerve, 9 (60%) had normal serum TG levels and 7 (63.6%) had high serum TG levels. The difference in percentage of serum TG levels in both groups was not statistically significant (p = 1.000).

The association between serum HbA1c levels and NCV in peroneal motor nerve was shown in table 2. In DM patients with polyneuropathy with low NCV in peroneal motor nerve, there was 1 subject (16.7%) whose serum HbA1c levels were <7% and 15 subjects (75%) whose serum HbA1c ≥ 7%. The percentage difference was statistically significant with p = 0.018. The value of odd ratio was 15, with a confidence interval range of 1,397 - 161,045 (95% CI).

Table 1. The characteristics of research subjects

<table>
<thead>
<tr>
<th>HbA1c level</th>
<th>Total</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 7 %</td>
<td>&lt;7%</td>
<td>&gt;7%</td>
</tr>
<tr>
<td>N = 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (22.2%)</td>
<td>7 (77.8%)</td>
<td>9 (100%)</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 (23.5%)</td>
<td>13 (61.5%)</td>
<td>17 (100%)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>56.67 ± 6.861</td>
<td>56.90 ± 5.486</td>
<td>0.693</td>
</tr>
<tr>
<td>Period of suffering from DM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 10 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 (30.8%)</td>
<td>9 (69.2%)</td>
<td>13 (100%)</td>
</tr>
<tr>
<td>≥10 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (15.4%)</td>
<td>11 (84.6%)</td>
<td>13 (100%)</td>
</tr>
<tr>
<td>Serum TG level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 (26.7%)</td>
<td>11 (73.3%)</td>
<td>15 (100%)</td>
</tr>
<tr>
<td>High</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (18.2%)</td>
<td>9 (81.8%)</td>
<td>11 (100%)</td>
</tr>
</tbody>
</table>
Table 2 The association between gender and nerve conduction velocity (NCV) in peroneal motor nerve

<table>
<thead>
<tr>
<th></th>
<th>NCV in peroneal motor nerve</th>
<th>Total n= 26</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal (N = 10)</td>
<td>Low (N = 16)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>3 (33.3%)</td>
<td>6 (66.7%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>7 (41.2%)</td>
<td>10 (58.8%)</td>
<td></td>
</tr>
<tr>
<td>Period of suffering from DM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 10 years</td>
<td>5(38.5%)</td>
<td>8(61.5%)</td>
<td>13 (100%)</td>
</tr>
<tr>
<td>≥10 years</td>
<td>5(38.5%)</td>
<td>8(61.5%)</td>
<td>13 (100%)</td>
</tr>
<tr>
<td>Serum TG level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>6 (40%)</td>
<td>9 (60%)</td>
<td>15 (100%)</td>
</tr>
<tr>
<td>High</td>
<td>4(36.4%)</td>
<td>7(63.6%)</td>
<td>11 (100%)</td>
</tr>
<tr>
<td>Serum HbA1c level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 7%</td>
<td>5(83.3%)</td>
<td>1 (16.7%)</td>
<td>6 (100%)</td>
</tr>
<tr>
<td>≥7%</td>
<td>5 (25%)</td>
<td>15 (75%)</td>
<td>20 (100%)</td>
</tr>
</tbody>
</table>

Discussion

In the basic data characteristics of research subjects, there was no significant difference in several basic variables including gender and age. It needs to be taken into account because gender and age affect HbA1c values. A study revealed that males had a greater prevalence of DM than females (7.5% males, 3.4% females, p <0.001). However, in elderly (> 66 years), it occurs in more females. Long-suffering from DM also affects HbA1c levels, as stated in the study that HbA1c levels significantly increased with duration of DM. In addition, it is stated that hypertriglyceridermia may decrease HbA1c. Age and gender can affect nerve conduction velocity, as stated that subjects with elder age have longer latency, smaller amplitude, and slower nerve conduction velocity than young subjects 12.

Females have a higher amplitude at the examination in the upper extremities, shorter latency of upper extremities, and longer latency in lower extremities than males. Long-suffering from DM also affects the velocity of the nerve conduction, as it is stated that the occurrence of a microvascular complication of DM along with the duration of DM is associated with low nerve conduction velocity 13. Triglyceride levels can also indirectly affect the velocity of nerve conduction. Hyperlipidemia is important in the development of diabetic polyneuropathy, as elevated serum triglyceride level correlates with decreased myelin fiber density which is independent of other variables such as age, DM length, DM control, and other variables. Therefore, this study attempted all subjects in relatively similar conditions, with the expectation of minimizing the influence of other factors that will affect the value of the variables studied 14.

Other factors that affect HbA1c are anemia, impaired renal function, alcohol addiction as well as factors affecting nerve conduction velocity, such as kidney function impairment and alcoholism have been attempted to be controlled by researchers through several components of exclusion criteria. The results of this study statistically obtained a significant association between serum HbA1c levels with peroneal motor nerve conduction velocity. In DM patients with polyneuropathy
with low peroneal motor nerve conduction velocity, there was 1 subject (16.7%) whose serum HbA1c levels were <7% and 15 subjects (75%) with serum HbA1c levels ≥7%. The percentage difference was statistically significant with $p = 0.018$. The results of this study also obtained the value of Odds ratio of 15 with the range of confidence interval 1.397 - 161.045; thus, it is concluded that serum HbA1c ≥ 7% is a risk factor for low peroneal motor nerve conduction velocity.\(^\text{15}\)

It is in accordance with a study by Shekharappa et al., which mentions that there is progressive neuronal damage in DM patients triggered by poor blood glucose control, in which the researchers compared the nerve conduction velocity between DM patients and non-DM patients.\(^\text{16}\) The wide range of confidence intervals in this study could be due to small sample size or basic data characteristics such as long-term suffering of DM, ranging from 1 year to 20 years, although in the analysis, the duration of DM is categorized into two groups.\(^\text{17}\)

**Conclusion**

There was an association between blood glucose level control measured by serum HbA1c levels with peroneal motor nerve conduction velocity in type 2 DM patients with polyneuropathy.

**Ethical Clearance:** This research involves participants in the survey using sampling method that was accordant with the ethical research principle based on the regulation of research ethic committee. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, nonmaleficeince, and justice.

**Conflict of Interest:** The authors swear that there is no conflict of interest related with this paper.

**Source of Funding:** This study is done with authors’ funding only.

**References**


List of Tables
Decrease of Sensorineural Hearing Frequency and Cochlear Hair Cell Function in Malignant Head and Neck Tumor Post Cisplatin Chemotherapy Series II

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1Departement of Otolaryngology, Faculty of Medicine, Universitas Airlangga-Dr. Soetomo General Hospital Surabaya. 60131 Indonesia

Abstract

Background: Malignancy in head and neck is mostly squamous cell carcinoma which are relatively radiosensitive. Giving chemotherapy combination of cisplatin-5 fluorouracil most frequently used in Lotus inpatients unit of Dr. Soetomo General Hospital Surabaya.

Purpose: This study aimed to prove the decrease of sensorineural hearing frequency and cochlear hair cells function in malignant head and neck tumors patients post-cisplatin chemotherapy series II.

Method: The samples were malignant head and neck tumor patients that would receive cisplatin chemotherapy. The inclusion criteria were aged 18 to 65 years, malignant head and neck tumors planning to get cisplatin chemotherapy, intact tympanic membrane, ANM checks within normal limits. Tympanogram type A, DPOAE examination with pass results.

Results: Pre-and post-cisplatin chemotherapy series I did not obtain any changes, those were pass on all frequencies. Mc Neymar test on DPOAE post-cisplatin chemotherapy I obtained p = 0.250 in 6 kHz frequency (p>0.05). DPOAE post-cisplatin II in 6 kHz frequency showed no significant difference (p>0.05)

Conclusion: There was no decrease of outer cochlear hair cells function in malignant head and neck tumors patients who received cisplatin chemotherapy series II.

Keywords: cisplatin-5 fluorouracil chemotherapy, sensorineural hearing, intact tympanic membrane, cochlea.

Introduction

Head and neck malignancy is a major health problem in the world. In America 2006 it is estimated about 500,000 new cases1. Head and neck malignancy (>75%) is a type of squamous cell carcinoma which is relatively radiosensitive. Approximately one-third is still in early stage that can be treated with surgery or radiotherapy. Therefore chemotherapy is an alternative choice in the treatment of malignant head and neck tumors2.

Combination chemotherapy of cisplatin-5 fluorouracil is frequently used in Lotus inpatients unit of Dr. Soetomo General Hospital Surabaya3. Along with this fact, chemotherapy toxicity is now a concern. one of them is ototoxic. Chemotherapy drugs that have ototoxic effects are cisplatin, carboplatin, nitrogen mustard, metrotrexat, vincristine, dactinomicyn, and bleomicyn4.

Cisplatin (cis-diamminedichloroplatinum-CDPP) is one of the most widely used and most potent chemotherapeutic drugs for malignant head and neck tumors treatment. Giving high doses of cisplatin 100-120 mg/m² of body surface could cause progressive sensorineural hearing loss. Irreversible, and bilateral starting at a frequency above 8000 Hz which will eventually affect the lower frequencies if therapy is continued and accompanied by tinnitus5. A research in America reported that cisplatin chemotherapy led to sensorineural hearing loss of 82.6% at a frequency of
3 kHz, 45.3% at 4 kHz, 81.0% at 6 kHz and 90.5% at 8 kHz with pure tone audiogram examination. Another study on malignant tumors patients using Distortion Product Otoacoustic Emissions (DPOAE) obtained outer hair cell damage of 63.4% (6.7% at 6-10 kHz, 56.7% at 8-10 kHz) post-first chemotherapy and 70% post-second chemotherapy (8.3% at 4-10 kHz, 31.7% at 6-10 kHz, 30% at 8-10 kHz). Hearing loss at high frequency may not cause communication disorder but continuously there will interfere conversations frequency resulting patient’s communication disorder. Cisplatin damages the cochlear outer hair cells progressively from the base to the apex therefore sensorineural hearing loss occurs starting at high frequency. Apoptosis causes death of cochlear outer hair cells resulting in sensorineural hearing loss. On the condition of outer hair cells damaged, the damage can affect the inner hair cells and supporting cells. The cisplatin ototoxicity is not limited to the cochlear outer hair cells but also reported to occur atrophy of vascular stria, collapse of reisnerr membrane and the degeneration of spiral ganglion.

Cochlear hair cells play a role in improving or sharpening the peak of traveling waves by increasing basilar cellular activity on a particular frequency. Cochlear outer hair cells damage due to cisplatin occurs from the third day in the basal to apex. An objective diagnose tool of cochlear outer hair cell damage is otoacoustic emission. Distortion product otoacoustic emission can evaluate cochlear response at high frequency which is the sensitive frequency to detect muscle ototoxicity.

**Method**

This study was an observational study with a non-comparable longitudinal approach conducting at Audiology inpatient unit of Otolaryngology Department Faculty of Medicine, Universitas Airlangga-Dr. Soetomo General Hospital Surabaya, Indonesia. The samples were malignant head and neck tumor patients who would receive cisplatin chemotherapy. The sampling technique used in this study was consecutive sampling.

The inclusion criteria were age 18 to 65 years, malignant head and neck tumors planning to receive cisplatin chemotherapy, intact tympanic membrane, ANM checks within normal limits, Tympanogram type A, and DPOAE examination with pass results. While the exclusion criteria were presence history of noise exposure, radiotherapy in the temporal bone area, previous otic drugs, hypertension and diabetes mellitus.

This research used the following tools and materials; tools for ear examination consisting of head lamp, cotton wool, cotton and otoscope. ANM checking tool consisted of Audiometer AD type 229E, a tympanometry type Inter acoustic AT 35. DPOAE examination tool used 100 mg/m^2^ body surface and data collection sheet.

The samples were patients with tympanogram A. This study used one ear sample but if both ears met the criteria, the sample used was right ear. ANM and DPOAE I (pre chemotherapy)/cisplatin chemotherapy I used 100 mg/m^2^ body surface. After 3 days, ANM and DPOAE II (post-chemotherapy I) were performed. Administration of cisplatin chemotherapy II was given 3-4 weeks after cisplatin chemotherapy I. After 3 days of cisplatin II series, ANM and DPOAE III (post cisplatin II chemotherapy) were performed.

The data were tabulated and analysed statistically using SPSS (SPSS. Inc. Chicago IL). The data obtained was then examined and processed statistically by paired sample t test to analyze high frequency sensorineural hearing loss and McNemar test to analyze the decrease of cochlear outer cell function pre and post cisplatin chemotherapy.

**Result**

Table 1 showed age, sex and diagnosis distribution.

**Table 1. Age, Sex and Diagnosis Distribution**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
A paired sample t test on ANM results post-cisplatin chemotherapy I and II obtained \( p = 0.339 \) at 4 kHz, \( p = 0.053 \) at 6 kHz and \( p = 0.809 \) at 8 kHz that meant ANM post cisplatin chemotherapy I and II has no significant difference (\( p > 0.05 \)). Based on statistical calculation using paired sample t test obtained \( p = 0.339 \) at 4 kHz, \( p = 0.002 \) at 6 kHz and \( p = 0.166 \) at 8 kHz that meant ANM pre and post cisplatin chemotherapy II at 4 kHz and 8 kHz had no significant difference (\( p > 0.05 \)) while at 6 kHz had a significant difference (\( p < 0.05 \)). DPOAE pre-and post-chemotherapy cisplatin I obtained pass at all frequencies, therefore the data obtained were homogeneous and could not be done a statistical test. DPOAE examinationpre-chemotherapy was 12 patients (100%) and 12 patients (100%) post-chemotherapy (Table 2).

### Table 2. ANM Result

<table>
<thead>
<tr>
<th>ANM</th>
<th>Pre-Chemotherapy</th>
<th>Chemotherapy I</th>
<th>Changes</th>
<th>T</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>SD</td>
<td>Average</td>
<td>SD</td>
<td>Average</td>
</tr>
<tr>
<td>4 KHz</td>
<td>22.50</td>
<td>5.000</td>
<td>22.50</td>
<td>5.000</td>
<td>-</td>
</tr>
<tr>
<td>6 KHz</td>
<td>22.08</td>
<td>6.557</td>
<td>24.17</td>
<td>6.86</td>
<td>-2.083</td>
</tr>
<tr>
<td>8 KHz</td>
<td>22.92</td>
<td>3.965</td>
<td>24.17</td>
<td>4.174</td>
<td>-1.250</td>
</tr>
<tr>
<td>ANM Result Pre and Post Cisplatin Chemotherapy I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANM Result Post Cisplatin Chemotherapy I and II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
McNemar test obtained $p = 0.250$ at 6 kHz while at 1, 2, 3 and 4 kHz, DPOAE results were pass, no changes post-cisplatin chemotherapy I and II (homogeneous), thus the data could not be tested. DPOAE post-cisplatin chemotherapy I and II at 6 kHz had no significant difference ($p>0.05$). McNemar test obtained $p = 0.250$ at 6 kHz while at 1, 2, 3 and 4 kHz, DPOAE results were pass, no changes post-cisplatin chemotherapy II (homogeneous). DPOAE post-cisplatin chemotherapy II at 6 kHz showed no significant difference ($p>0.05$).

**Table 3. DPOAE Result**

<table>
<thead>
<tr>
<th>DPOAE</th>
<th>Pre</th>
<th>Post I</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPOAE Result Pre and Post Cisplatin Chemotherapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pass (1.2.3.4.6 kHz)</td>
<td>12 (100%)</td>
<td>12 (100%)</td>
</tr>
<tr>
<td>Refer (1.2.3.4.6 kHz)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

| DPOAE Result Post Cisplatin Chemotherapy I and II | | |
| Pass freq. 1.2.3.4.6 kHz | 12 (100%) | 9 (75%) |
| Refer freq. 1.2.3.4 kHz freq. 6 kHz | - | - |
| 3 (25%) * | |

| DPOAE Result Pre and Post Cisplatin Chemotherapy II | | |
| Pass freq. 1.2.3.4 kHz | 12 (100%) | 9 (75%) |
| Refer freq. 1.2.3.4 kHz Freq. 6 kHz | - | - |
| 3 (25%) * | |

**Discussion**

The results indicated that the most patients based on age distribution aged 35-45 years. Most patients with nasopharyngeal carcinoma were on 4-6th decade and the climax was on 5th decade that was between 41-50 years. In 2009, most sinonasal carcinoma patients in Dr. Soetomo General Hospital aged>40 years. Age over 50 years is very influential on the incidence of malignant head and neck tumor due to a decrease in physiological capacity and reduced ability to deal with environmental stress.
The ratio of male versus female was 2:1. This result was in accordance with the data of Oncology Unit of Otolaryngology Department, Dr Soetomo General Hospital that nasopharyngeal carcinoma was the most malignant head and neck tumor. The ratio of male versus female were 2:1 in nasopharyngeal carcinoma, 3:2 in sinonasal carcinoma, and 11:1 in laryngeal carcinoma12,13. Male is the most suspected gender because they often go out of the house to work therefore the risk of carcinogenic substances exposure and other environmental factors are higher1.

ANM pre and post cisplatin chemotherapy I at 4 kHz had no changes therefore the data could not tested statistically. While at 6 and 8 kHz obtained no significant different. This was different with other studies that obtained sensorineural hearing loss as much as 33% post-cisplatin chemotherapy I but it was not stated at what frequency the sensorineural hearing loss happened15. This difference could also be affected by the effects of chemotherapy combined with radiotherapy, thus increasing toxicity. This study was consistent with another study that stated the cisplatin ototoxicity with conventional ANM examination obtaining 1 person (35.7%) with sensorineural hearing loss, if with the extended high frequency (EHF) audiometry obtaining 88.1% with the range of 40-60 dB16.

The researchers have not been able to find the reason why the significance only occurred at 6 kHz while at 4 kHz and 8 kHz pre and post cisplatin chemotherapy II obtained no significance difference. This result was inconsistent with other studies suggesting sensorineural hearing loss occurring since the administration chemotherapy and being progressive with larger cumulative doses15. Based on comparative studies between audiogram and cochlear damage, cochlear outer hair cell damage caused hearing loss between 55-65 dB. Cochlear inner hair cells caused a very heavy hearing loss of 95 dB14.

There was no decrease in cochlear outer hair cell function in patients with head and neck tumor post-cisplatin chemotherapy I. This was not in accordance with the theory that cisplatin would cause disturbance after cisplatin chemotherapy I. Other studies showed the results of DPOAE post-cisplatin chemotherapy I obtaining refer at 6 kHz frequency as much as 6.7% and at 8 kHz as much as 56.7%. Another difference was the number of samples of 30 people that was more than this study7.

DPOAE post-cisplatin chemotherapy I and II obtained no significant difference. This is different from other research obtaining refer at 4 kHz frequency as much as 8.3%, 6 kHz as much as 31.7% and 8 kHz as much as 30%7. This difference could be due to the differences of sample size and the weakness of the tools used in this study. Research on Testicular Carcinoma in Hungary showed SNHL in samples receiving cumulative doses >400 mg/m² while in samples with cumulative doses <300 mg/m² obtained no significant difference17.

Pre and post cisplatin chemotherapy II results obtained no significant difference. The cisplatin ototoxicity was highly dependent on cumulative doses. Ototoxicity increased at doses >400 mg, with DPOAE examination obtained referral data at lower frequencies. In this study chemotherapy I used accumulative dose between 120-170 mg, post-chemotherapy II used cumulative dose between 240-340 mg. Research on testicle carcinoma in Hungarian obtained an impaired function of cochlear outer hair cells in samples receiving cumulative doses >400 mg/m² while in samples with cumulative doses <300 mg/m², there was no significant impaired function of cochlear hair cells17. This inadequacy of this study with other studies might be due to several factors that induce cisplatin ototoxicity. Many things could be an induced factors of cytotoxicity such as cumulative dose (>400 mg), long-term administration (>6 months), administration method, individual susceptibility variation, extreme age, previous hearing loss, anemia, radiation history, and the use of other ototoxicity drugs17. Chemotherapy affects the body cells. Continuous exposure causes damage to hair cells in the cochlea that cause cochlear dysfunction and will increase the hearing threshold18. Chemotherapy has physical and psychological side effects19. There was a correlation between the increased dose of cisplatin with the cytotoxicity effects on NPC stem cell20.

Conclusion

It was obtained sensorineural hearing loss at 6 kHz frequency, while at 4 and 8 kHz frequencies were still in normal state (no hearing loss). Malignant head and neck tumor patients who received cisplatin chemotherapy II. There was no decrease in cochlear outer hair cells in malignant head and neck tumor patients who received cisplatin chemotherapy II.

Ethical Clearance: This research is approved by Ethical Clearance from Universitas Airlangka dan Dr
Soetomo Teaching Hospital

**Conflict of Interest**: There is no conflict of interest reported from this research.

**Source of Funding**: This research funded individually.

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Thiophene-Cyclic and Sulfazane Derivatives (Preparation, Spectral Analysis, the Behavior in Organic Solvents, Microbial Testing)

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Abstract

The thiophene core is the most broadly applied constructing blocks to the production of many biomolecules, pharmaceutical drugs, vitamins, also in industrial compounds, in dyes compounds because of its high stability in environment. Thiophene derivatives were synthesized in present study from chalcone compound as a starting material from aldol reaction in first step, followed by cyclization reactions by using various conditions, and this paper involved new reaction for diazonium salts to producing (Sulfide-azo) compounds in same bond for the first time that we termed (Sulfazane) as a first and Novel preparation to this new type of compounds., the synthesized derivatives were confirmed through numerous spectral techniques (FT.IR, H.NMR, C.NMR), other physic-chemical data for synthesized thiophene derivatives, besides to studying of behavior of compounds in organic solvents and screening tests for efficiency of thiophene derivatives against microbes.

Keywords: Microbial, Sulphazane, Aldol, chalcone, thiophene, heterocyclic, phenylene diamine, coupling, Azo, new reaction of diazonium, sulfide-azo, (-S-N=N-), seven membered, five membered.

Introduction

Thiophene is a colorless liquid in a benzene-like odor., with aromatic properties involving one hetero atom (sulfur) in the formula structure(C₄H₄S), including a planar five-membered ring (four carbon atoms and one sulfur atom), it behaves in the reactions as a substitution reaction. Thiophene is stable in high temperatures and its nucleus in most natural molecules like biotin, numerous plant colors, natural dyes, besides various pharmaceuticals., some thiophene derivatives have applications in antifungal fields.

Experimental Part

The routes of reaction carried out by many steps, various conditions, different catalysts to formation heterocyclic compounds from chalcone-thiophene, all formatted derivatives followed with (TLC) in addition to iodine vapor chamber. Thiophene derivatives {1-8} were established via various apparatuses: FT-IR spectra (FT-IR 8300 Shimadzu) with the range (400-4000)cm⁻¹ using discs of KBr., ¹H.NMR–Spectra besides ¹³C.NMR with solvent (d-DMSO), besides to studying of behavior of compounds in organic solvents and microbial screening:

Routes of Preparation⁰⁻¹⁵:

Route of Preparation for Thiophene Derivatives {1, 2, 3}

P-Formal benzoic acid(0.01 mole) rotated with 2-acetothiophene (0.01 mole) at room temperature via mechanical rotation for (8 hrs) according to processes in basic medium to yield product that acts chalcone Compound{1}, the next step, drying, re crystallized
weigh (0.01 mole) cyclized with (0.01 mole) of ortho-aminophenol in refluxing step, the resulting filtered, dried followed by re-crystallized to give seven-membered ring from compound{2}, which (0.01 mole) condensed for (26 hrs) with (0.01 mole) of thiosemicarbazide in basic medium (5% NaOH) in two steps according to processes\(^{8-12}\), then filtered, dried, then recrystallized to obtain triazole derivative as a compound{3}.

The resulting compound washed in distilled water, dried, to give (S-N=N-)Sulfazane compound {4}.

Compound{3} (0.01 mole) reacted in basic medium via coupling reaction with (0.01 mole) of p-methyl phenyl diazonium salt-derivative at (0-5) C° according to approaches\(^9,15\), after (24 hrs) . The resulting compound washed in distilled water, dried, recrystallized to give (S-N=N-) Sulfazane compound {5}.
Route of Preparation for Thiophene Derivatives{6,7,8}

Thiophene Compound{2}(0.01 mole) cyclized with (0.01 mole) of ortho-phenyl diamine in refluxing step in (4N of HCl) in refluxing process according to approaches(9-12), the resulted compound filtered, dried followed by re-crystallized to produce compound {6}.

Thiophene Compound {2}(0.01 mole) cyclized with (0.01 mole) of ortho-aminophenol in refluxing step in (4N of HCl) in refluxing process according to approaches(9-12), the resulted compound filtered, dried followed by re-crystallized to produce compound {7}.

Thiophene Compound {2}(0.01 mole) cyclized with (0.01 mole) of ortho-thiol aniline in refluxing step in (4N of HCl) in refluxing process according to approaches(8-10), the resulted compound filtered, dried followed by re-crystallized to produce compound {8}.

Results and Discussion:

The products of thiophene compounds prepared via cyclization reaction of chalcone compound which prepared in previously step (aldol reaction)(14), followed by cyclization with amino derivative to yield seven membered ring, then cyclization reaction with thiosemicarbazide to yield triazole derivative with thiol (SH)- terminal that reacted with various diazonium salts–derivatives in new reaction step in this paper by (Dr. Nagham Aljamali –as a new reaction for diazonium salt) that we termed (Sulfazane) as a first and novel preparation to this new type of compounds.

All Thiophene-heterocyclic and sulfide-azo compounds examined by spectral characterization besides microbial screening:

Spectral Description:

**FT-IR- Description of Spectra** : From spectral analysis, that noted disappearance of bands while appearance of new bands to indicate to formation of Thiophene-cyclic and that improve formation of (sulfide–Azo) compounds as a new band and as evidence for new reaction:

Thiophene –Chalcone Compound {1}: bands at (-CO-O) carboxyl of carboxyl group: 1706, (CO-) carboxyl of chalcone: 1667, (C-S) thiophene: 753, (=CH-) Alkene: 3049.


Thiophene (Sulfide- Azo) Compound {4}: new bands improved formation of (sulfide–Azo) (S-N=N-).
at (1389, 1454, 1515) as a new band in new reaction .
(C-O-C): 1134, (C-S) thiophene : 779, (=CH-) Alkene :
3067, (C=N) endocycle: 1657, (NH) amine : 3273, (CH)
aliphatic : 2985.

Thiophene (Sulfide- Azo) Compound {5}: new
bands improved formation of (sulfide–Azo) (S-N=N-)
at (1384, 1467, 1509), (C-O-C): 1165, (C-S) thiophene :
786, (C=H-) Alkene: 3092, (C=N) endocycle: 1655, 
(NH) amine : 3256, (CH) aliphatic : 2976.

Thiophene –imidazole Compound {6}: bands at
(C-O-C): 1155, (C-S) thiophene : 769, (C=H-) Alkene :
3071, (C=N) endocycle: 1642, 1663, (NH) in imidazole :
3310.

Thiophene –Oxazole Compound {7}: bands at
(C-O-C): 1147, (C-S) thiophene : 791, (C=H-) Alkene :
3069, (C=N) endocycle: 1639, 1658, (C-S-) Oxazole :
1189.

Thiophene –Thiazole Compound {8}: bands at
(C-O-C): 1147, (C-S) thiophene : 791, (C=H-) Alkene :
3069, (C=N) endocycle: 1635, 1650, (C-S-) in Thiazole :
763.

Other functional groups appeared in some spectra
figure(1).

1H.NMR- Description of Spectra: From spectral
analysis, that noted disappearance of peaks while
appearance of new peaks to indicate to formation of
Thiophene-cyclic and (sulfide–Azo) compounds, all
spectra appeared signal at (2.50) due to solvent (d-
DMSO), and other signals like:

Thiophene–Chalcone Compound {1}: appearance
signals at (12.66) due to proton of carboxyl group
(COOH), (6.14, 6.33) to protons of chalcone (CO-
CH=CH-), (6.63 – 7.99) to protons of aromatic ring.

Thiophene–Seven Membered ring Compound {2}:
appearance signals at (12.20) due to proton of carboxyl
group (COOH), (2.83) to proton of alkene (-C=CH-),
(6.65 – 7.51) to protons of aromatic ring.

Thiophene–Triazole Compound {3}: appearance
signals at (8.24) due to proton of amine in Triazole ring
(NH), (13.54) due to proton of Thiol group (SH), (2.64)
to proton of alkene (-C=CH-), (6.72 – 7.67) to protons
of aromatic ring.

Thiophene (Sulfide- Azo) Compound {4}: appearance
signals at (8.19) due to proton of amine in
Triazole ring (NH), (0.98) due to protons of methyl
groups (CH3), (2.68) to proton of alkene (-C=CH-),
(6.90 – 7.74) to protons of aromatic ring.

Thiophene (Sulfide- Azo) Compound {5}: appearance
signals at (8.27) due to proton of amine in
Triazole ring (NH), (0.63) due to protons of methyl
group (CH3), (2.72) to proton of alkene (-C=CH-),
(6.85 – 7.81) to protons of aromatic ring.

Thiophene –Imidazole Compound {6}: appearance
signals at (8.11) due to proton of amine group (NH) in
Imidazole, (2.81) to proton of alkene (-C=CH-),
(6.77 – 7.67) to protons of aromatic ring.

Thiophene –Oxazole Compound {7}: appearance
signals at (2.64) to proton of alkene (C=CH-),
(6.95 – 7.87) to protons of aromatic ring.

Thiophene –Thiazole Compound {8}: appearance
signals at (2.61) to proton of alkene (C=CH-),
(6.90 – 7.73) to protons of aromatic ring.

Other protons of functional groups appeared in
some spectra figure (1).

The 13C.NMR Description of Spectra: From
spectral analysis, that noted disappearance of peaks while
appearance of new peaks to indicate to formation of
Thiophene-cyclic and signals that improve formation of
(sulfide–Azo) compounds, all spectra appeared signal
at (40.00) due to solvent (d-DMSO), and other signals
like:

Thiophene–Chalcone Compound {1}: appearance
peaks at (171.04) due to carbon atom of (COOH)
carboxyl group, (110.0, 112.1) due to carbon atoms
of chalcone (-CH=CH-), (179.7) due to carbonyl in
chalcone (-CO-), (120–132) due to carbon atoms of
aromatic ring, (136–142) due to carbon atoms of
heterocycles.

Thiophene–Seven Membered ring Compound {2}:
appearance peaks at (171.05) due to carbon atom of
(COOH) carboxyl group, (100.0, 105.7) due to carbon
atoms of alkene (-CH=CH-), (112–127) due to carbon atoms
of aromatic ring, (132–144) due to carbon atoms of
heterocycles.

Thiophene–Triazole Compound {3}: appearance
peaks at (101.22, 104.83) due to carbon atoms of alkene
(-CH=C-), (152.12) due to carbon atom of
(-C=N-), (116–130) due to carbon atoms of aromatic
ring.
Thiophene (Sulfide- Azo) Compound \{4\}: appearance peaks at (100.14, 103.92) due to carbon atoms of alkene (-CH=C-), (150.36) due to carbon atom of (-C=N-), (111.32 – 129.08) due to carbon atoms of aromatic ring, (133.8 – 148.75) due to carbon atoms of heterocycles, (17.45, 19.61, 19.92) due to carbon atoms of methyl groups.

Thiophene (Sulfide- Azo) Compound \{5\}: appearance peaks at (100.76, 102.38) due to carbon atoms of alkene (-CH=C-), (149.52) due to carbon atom of (-C=N-), (115.76 – 130.17) due to carbon atoms of aromatic ring, (134.41 – 146.27) due to carbon atoms of heterocycles, (16.96) due to carbon atom of methyl group.

Thiophene – imidazole Compound \{6\}: appearance peaks at (101.54, 103.21) due to carbon atoms of alkene (-CH=C-), (150.56) due to carbon atom of (-C=N-), (109.73–130.94) due to carbon atoms of aromatic ring, (135.87–146.89) due to carbon atoms of heterocycles.

Other carbon atoms of functional groups appeared in some spectra, figure (1).

Fig(1): Spectra of Antimicrobial Compounds\{1,2\}

Behavior of Thiophene Derivatives in Organic Solvents
Thiophene–Cyclic and Sulfide- Azo (Sulphazane compounds) screened and tested in various organic solvents, the solvents that selected have various polarities for this reason, some of prepared thiophene compounds have solubility in polar solvents while other compounds have no solubility in these solvents, Table (1).

**Table 1: Properties of Thiophene Derivatives and Solubility**

<table>
<thead>
<tr>
<th>Compds</th>
<th>Product %</th>
<th>Color</th>
<th>M.P (°C)</th>
<th>Solubility in solvents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ethyl Alcohol</td>
</tr>
<tr>
<td>{ 1 }</td>
<td>80</td>
<td>Yellow</td>
<td>168</td>
<td>+</td>
</tr>
<tr>
<td>{ 2 }</td>
<td>74</td>
<td>Deep Yellow</td>
<td>176</td>
<td>+</td>
</tr>
<tr>
<td>{ 3 }</td>
<td>70</td>
<td>Yellowish Brown</td>
<td>184</td>
<td>+</td>
</tr>
<tr>
<td>{ 4 }</td>
<td>72</td>
<td>Deep Yellow</td>
<td>230</td>
<td>+</td>
</tr>
<tr>
<td>{ 5 }</td>
<td>70</td>
<td>Yellowish Orange</td>
<td>218</td>
<td>+</td>
</tr>
<tr>
<td>{ 6 }</td>
<td>68</td>
<td>Orange</td>
<td>190</td>
<td>+</td>
</tr>
<tr>
<td>{ 7 }</td>
<td>64</td>
<td>Reddish Orange</td>
<td>194</td>
<td>+</td>
</tr>
<tr>
<td>{ 8 }</td>
<td>70</td>
<td>Yellowish Orange</td>
<td>200</td>
<td>+</td>
</tr>
</tbody>
</table>

**Assay of Bacterial Growth**

The antimicrobial activity was tested via incubation of two types of bacteria anaerobically at 37°C for (48 hr) by using three concentrations of each plate was measured for the zone of inhibition (diameters of the discs to the nearest whole number), Table (2).

![Fig(2):Selected Bacteria](image-url)
Table 2: Antibacterial Assay of Thiophene compounds in Concentration (10 µ .gm)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Bacillus subtilis</th>
<th>Staphylococcus epidermidis.</th>
</tr>
</thead>
<tbody>
<tr>
<td>{ 1 }</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>{ 2 }</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>{ 3 }</td>
<td>14</td>
<td>16</td>
</tr>
<tr>
<td>{ 4 }</td>
<td>Sulfazane</td>
<td>18</td>
</tr>
<tr>
<td>{ 5 }</td>
<td>Sulfazane</td>
<td>16</td>
</tr>
<tr>
<td>{ 6 }</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>{ 7 }</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>{ 8 }</td>
<td>14</td>
<td>14</td>
</tr>
</tbody>
</table>

The results indicated to high inhibition for (Sulfide–Azo) derivatives {4,5} than other thiophene derivatives due to new band sulfide linked with azo group(S–N=N–) which gave high efficiency in decreasing resistance of bacteria towards these new compounds (Sulfide- Azo).

**Conflict of Interest**: There is no any Conflict of Interest

**Ethical Clearance**: Ethics committee refer that there is no plagiarism and there is no mistakes or wrong results in this work.

**Source of Funding**: Self funding.

**References**

12. Nagham M A. “Synthesis and Biological Study


Diagnostic test of Brief Peripheral Neuropathy Screen as Distal Sensory Polyneuropathy-HIV Diagnostic Tool

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Abstract

Distal sensory polyneuropathy (DSP) is the most common human immunodeficiency virus (HIV)-associated peripheral neuropathy with a prevalence of 30-67%. Brief peripheral neuropathy screen (BPNS) examination is a non-invasive, fast, cheap, easy-to-do diagnostic method, and one of the clinical tools that can be used to diagnose HIV-DSP. This study to assess the BPNS diagnostic test as a DSP diagnostic tool in HIV patients. The cross-sectional study was conducted on HIV patients that allegedly having DSP. All subjects were examined by BPNS and nerve conduction study (NCS), then the results were analyzed by chi-square test of a 2x2 Table. There were 42 subjects enrolled in this study. The mean patients were 38.88±8.62 years and most of the male patients were 24 subjects (57.14%). Most of the patients had high school education by 20 (47.61%) subjects and did not take anti-retroviral (ARV) by 18 (42.85%) subjects. Most of the research subjects conducted BPNS disturbed examination by 27 (64.28%) subjects and examination of NCS obtained positive DSP by 30 (71.4%) subjects. The obtained sensitivity of 83% BPNS, 83% specificity, 93% positive predictive value, the negative predictive value of 67%, a positive likelihood ratio of 5.00, and a negative likelihood ratio of 0.20. BPNS examination with 83% sensitivity and 83% specificity can be used as a DSP diagnostic tool in HIV patients.

Keywords: Diagnostic testing, DSP-HIV, Brief peripheral neuropathy screen (BPNS), NCS examination

Introduction

Human immunodeficiency virus (HIV) is a retrovirus that can cause immune system failure in humans 1,2. It is estimated that 35.3 (32.2-38.8) million people are living with HIV in 2012 and annually about 2.3 (1.9-2.7) million with new infections 3. After the highly active anti retroviral therapy (HAART) era since 1996, the life expectancy of HIV patients has increased. As a consequence, patients and medical personnel will be faced with neurological complications of HIV disease from comorbidities and the drugs used 1,4,5. Peripheral neuropathy has been documented since early reports of HIV disease and HIV isolation in the human nervous system. The prevalence of peripheral neuropathy in HIV-positive individuals are high and is the most common neurologic complication of HIV infection. Although not life-threatening peripheral neuropathy will affect the quality of life in HIV patients 1,4,6–9.

Distal symmetrical polyneuropathy (DSP) is the most common form of HIV-associated peripheral neuropathy, it also called distal sensory polyneuropathy. Two potential mechanisms for the cause of neurotoxicity plays an important role in the pathogenesis of HIV DSP: viral neurotoxicity and its products and neurotoxic side effects of treatment used in the managements of HIV 1,9. Where the prevalence ranges from 30%-67% 10. Until now there is no standard for diagnosing DSP-HIV, but several clinical devices have been developed to assess this condition in both clinical and research practice. One of the most commonly used clinical devices is the brief peripheral neuropathy screen (BPNS) 1.
Brief Peripheral Neuropathy Screen is one of the clinical tools that can be used to diagnose DSP-HIV. Non-neurological clinicians such as general practitioners, trained nurses or therapists may use BPNS to diagnose peripheral neuropathy. BPNS has been validated as a screening device to determine the degree of polyneuropathy. This check includes subjective and objective information and takes only <10 minutes for its implementation. The diagnosis of sensory HIV neuropathy can be established if the patient shows ≥1 specific symptoms of BPNS and 1 other sign of decreased ankle/Achilles reflex and/or vibration perception.

The availability of electro myo graphy (EMG) tools in peripheral areas in Indonesia is limited, so research is needed to assess the usefulness of BPNS diagnostic tools in establishing the diagnosis of HIV-DSP. Based on the international research, the data showed that BPNS diagnostic tests can be used to help establish a diagnosis of HIV-DSP. Therefore the researchers tried to conduct research on the diagnostic test of BPNS examination compared to the examination of nerve conduction/NCS in HIV patients with DSP suspicion in Dr. Soetomo Teaching Hospital, Surabaya.

**Method**

The study subjects were HIV patients with allegedly of DSP who visited outpatient of UPIPI at Dr. Soetomo Teaching Hospital Surabaya, Indonesia. The subject inclusion criteria were HIV patients with DSP suspicion and aged 18 to 60 years. While, the subject exclusion criteria were, patients with type 2 Diabetes Mellitus, using neurotoxic drugs (metronidazole, chloramphenicol, vincristine, vinblastine, dapsone, isoniazid, and ethambutol), lower extremity amputations, and lower extremities contracture. The subjects who are willing to participate the research in advance fill out the form informed consent. And then, before this study the researcher conducted a test of ethics at Dr. Soetomo Teaching Hospital Surabaya Indonesia.

This study used a cross-sectional study design that conducted over 5 months. The instruments used in this study were data collecting sheets from interviews and reflect and Achilles reflex examinations, Riester reflex hammer tool for Reaction Achilles inspection, 128 Hz tuning fork tool for vibration check, NCS tool is EMG/NCV Cadwell, paper of NCS readings. The stages of this study examination of BPNS, subsequent examination of NCS, and all recording results were collected for subsequent data tabulation and statistical analysis.

The data obtained from the data collection sheet then expressed on the scale of the dichotomies, i.e., the nominal scale having 2 positive or negative values. The examination results of HIV patients suspected of having DSP with BPNS examination that was analyzed by the 2x2 table for the dichotomous values, the difference of clinical findings and BPNS positive compared to negative findings. Then, it was compared to NCS, and analyzed by diagnostic test to obtain sensitivity, specificity, positive suspicion value, negative suspicion value, positive possibility ratio, and possible negative ratio. Statistical analysis using SPSS 19 (SPSS, Inc., Chicago, IL) and paint maker program.

**Results**

The data collection were BPNS examination and NCS examination conducted within 5 months, from December 2014 to April 2015. The basic data of the patient was taken by direct interview with the patient and the BPNS examination was performed by UPIPI whereas the neural conduction examination was done in Electromyography (EMG) room at Department of Neurology Dr. Soetomo Teaching Hospital Surabaya, Indonesia.

The mean patients were 38.88±8.62 years and most of the male patients were 24 (57.14%). Most of the patients had high school education by 20 (47.61%) subjects and did not take anti retro viral (ARV) by 18 (42.85%) subjects. Most of the research subjects conducted by BPNS examination that disturbed of 27 (64.28%) subjects and examination of NCS obtained positive DSP by 30 (71.4%) subjects (Table 1).
Table 1. Characteristics of research subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>Research subjects n=42</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>Sex</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>24</td>
<td>57.14</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>18</td>
<td>42.86</td>
<td></td>
</tr>
<tr>
<td>Education</td>
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<tr>
<td>Elementary School</td>
<td>9</td>
<td>21.42</td>
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<tr>
<td>Junior High School</td>
<td>11</td>
<td>26.19</td>
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<tr>
<td>Senior High School</td>
<td>20</td>
<td>47.61</td>
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</tr>
<tr>
<td>College</td>
<td>2</td>
<td>4.78</td>
<td></td>
</tr>
<tr>
<td>The use of ARV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non using ARV</td>
<td>18</td>
<td>42.85</td>
<td></td>
</tr>
<tr>
<td>TDF, 3TC, EFV</td>
<td>12</td>
<td>28.57</td>
<td></td>
</tr>
<tr>
<td>TDF, 3TC, NVF</td>
<td>3</td>
<td>7.14</td>
<td></td>
</tr>
<tr>
<td>AZT, 3TC, NVF</td>
<td>9</td>
<td>21.44</td>
<td></td>
</tr>
<tr>
<td>ARV-use duration</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Non using ARV</td>
<td>18</td>
<td>42.85</td>
<td></td>
</tr>
<tr>
<td>&lt;3 months</td>
<td>11</td>
<td>26.19</td>
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<tr>
<td>&gt;3 months</td>
<td>13</td>
<td>30.96</td>
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<tr>
<td>BPNS</td>
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<td>Disturbed</td>
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<td>Normal</td>
<td>15</td>
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<tr>
<td>NCS</td>
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</tr>
<tr>
<td>DSP (+)</td>
<td>30</td>
<td>71.43</td>
<td></td>
</tr>
<tr>
<td>DSP (-)</td>
<td>12</td>
<td>28.57</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Tabulation of BPNS and NCS examination results

<table>
<thead>
<tr>
<th>NCS</th>
<th>Total</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>DSP (+)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BPNS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DSP (+)</td>
<td>25 (59.53)</td>
<td>2 (4.75)</td>
<td>27 (64.28)</td>
</tr>
<tr>
<td>DSP (-)</td>
<td>5 (11.90)</td>
<td>10 (23.82)</td>
<td>15 (35.72)</td>
</tr>
<tr>
<td>DSP (+)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>30 (71.43)</td>
<td>12 (28.57)</td>
<td>42 (100.00)</td>
</tr>
</tbody>
</table>

Cross tabulation between BPNS diagnosis and NCS results in the study subjects resulted in a similar diagnosis which 27 patients diagnosed with DSP and BPNS, 25 patients (92.6%) were positively diagnosed with DSP and NCS as well. Out of the 15 patients diagnosed with DSP and BPNS, 10 patients (66.7%) were diagnosed as negative DSP and NCS (Table 2).
Discussion

Brief Peripheral Neuropathy Screen is one of the clinical tools that can be used to diagnose DSP-HIV \(^1,11\). BPNS cannot distinguish the underlying cause of neuropathy but shows its sensitivity to small nerve fibers/pain fibers, indicating the presence of DSP. This check includes subjective and objective information and takes only <10 minutes for its implementation. The HIV diagnosis of SN can be established if the patient showed ≥1 specific symptoms of BPNS and 1 other sign of decreased ankle/Achilles reflex and/or vibration sensation \(^13,14\).

The data from this study indicated that BPNS examination may be used to establish the diagnosis of HIV DSP, however, did not replace the function of NCS in identifying other conditions similar to DSP such as lumbosacral radiculopathy or another polyradiculoneuropathy. NCS is also more appropriate as a predictor of clinical severity than BPNS.

Diagnostic tests were distinguished by their usefulness as a screening tool, to ascertain and exclude disease, monitor disease course, and determine prognosis. Choosing the right diagnostic check is not easy. The ideal diagnostic test is rare, a test that gives positive results on all sick objects and gives negative results on all not sick subjects. Almost on all types of diagnostic tests it is possible to obtain positive test results on healthy subjects (false positives) and negative results on sick subjects (apparent negative) \(^15,16\).

This diagnostic test study aims to determine the sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio of BPNS with standard nerve conduction examination/NCS. A diagnostic test can be to diagnose a disease or exclude a disease if it has a high sensitivity and specificity \(^15\). A high sensitivity diagnostic test is required to detect the presence of a disease, as more cases can be detected by the test. High specificity diagnostic tests are used to confirm the presence of a disease because the greater the specificity of a diagnostic test, the greater the likelihood that patients who do not have a disease are excluded by the test \(^16\).

The results of previous studies showed a more comparison of male patients i.e., 85% and 84% \(^17,18\). According to the data of HIV patients that undergoing treatment at outpatient UPIPI of Dr. Soetomo Teaching Hospital where the number of male patients 4-7 times more than women \(^19\). However, in other studies obtained more women 69.8%, 65.3%, and 67.5% \(^13,20,21\). The results of this study was not much different from previous studies that get an average age of 36.5 ± 9.3 years and mean age was 38.3 ± 10.8 in the HAART group and 35.5 ± 8.7 in the non-HAART group \(^17,21\).

In this study, 18 subjects (42.85%) had not received antiretroviral therapy, as most new subjects were diagnosed, no CD4 results and some research subjects were suspected of having other opportunistic infections. Type of antiretroviral drugs used research subjects using 3 kinds of combinations, namely: a. tenofovir, lamivudine, efavirenz, b. tenofovir, lamivudine, nevirapine, and c. zidovudine, lamivudine, nevirapine. This therapy is according to WHO 2006 first-line therapy for adult patients \(^19\). Among the antiretrovirals used by the study subjects, there was zidovudine which can cause peripheral neuropathy. The subjects taking antiretrovirals less than 3 months were 26.1% and 31% were taking antiretroviral drugs for more than 3 months. According to a previous study, the peak incidence of DSP HIV occurred on day 90 after the use of antiretrovirals\(^17\).

Sensitivity is the proportion of ailing subjects with positive (absolute positive) diagnostic test results overall sick subjects (false positives and apparent negative) or the possibility that the diagnostic test results are positive when performed on a group of disease subjects. Specificity is the proportion of healthy subjects who give negative (true-negative) diagnostic test results compared to all non-sick subjects (true negative and false positives) or the possibility that the diagnostic test results will be negative when performed on a group of healthy subjects \(^15\).

In previous studies, BPNS showed a sensitivity of 49% and a specificity of 88% for diagnosing HIV-DSP \(^12\). This estimated value is strongly influenced by the prevalence of disease, where the population in this study is hospital-based so that more suffer than healthy. Positive assumptions are important statistics in the diagnostic test \(^15\).

Another statistic of the diagnostic test is the likelihood ratio (LR), i.e., the likelihood of the sick subjects getting the same test result. The positive likelihood ratio is the proportion of the proportion of sick subjects who test positive with the proportion of healthy subjects who test positive. The negative likelihood ratio is the proportion between the proportion of sick subjects
who tested negative tests and healthy subjects who gave negative test results. In this study, there was a positive likelihood ratio of 5.00 (CI 95%, 1.40-17.90) and a negative likelihood ratio of 0.20 (CI 95%, 0.09-0.46). According to the literature, the ratio of probability ratios varies from 0 to infinity. The results of positive positive diagnostic tests provided a probability ratio value much greater than 1, strong negative test results will give a probability ratio value close to 0.15.

BPNS examination can be used as a diagnostic tool of DSP HIV and has an advantage if it is compared to the examination of nerve conduction/ NCS. Daily clinical use is faster, easier, cheaper, non-invasive, and can be performed by trained medical and paramedical workers.

Conclusion

In this study, the sensitivity of BPNS examination was 83% and specificity was 83%, positive predictive value 93%, and negative predictive value 67%, positive likelihood ratio 5.00 and negative likelihood ratio 0.20.

Ethical Clearance: This study protocol was approved by ethical clearance Dr.Soetomo teaching hospital, Surabaya, Indonesia

Conflict of Interest: The author reports no conflict of interest of this work.

Source of Funding: This study is done with individual funding.

References


Relation of Homocysteine With Malondialdehyde and Dyslipidemia in Type 2 Diabetic Patients with Coronary Artery Diseases

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Department of Chemistry, College of Science, University of Diyala, Baquba, Diyala, Iraq

Abstract

Diabetes mellitus (DM) is one of the most common chronic disorder widely prevalent throughout the world. DM is characterized by elevation of blood glucose (hyperglycemia). Hyperglycemia, with time, led to several serious macro and microvascular complications.

The purpose of study was to investigate the relation of Homocysteine (Hcy) with oxidative stress (malondialdehyde”MDA”) and dyslipidemia in type 2 diabetic patients with coronary artery diseases in comparison to patients of coronary artery diseases (CAD) without DM.

Methods: The present study included 60 patients of coronary artery disease. Patients divided into two groups: group1 = 30 coronary artery disease patients without diabetic and group2 = 30 coronary artery disease patients with type2 diabetes. Homocysteine was estimated by competitive ELISA test using commercially available kit. MDA was estimated by colorimetric method. Lipid profiles were determined by using commercial available kits.

Results: Plasma levels of Hcy and MDA in group1 and group2 were significantly higher (p<0.05) than controls. In group1 Hcy shows significant (P<0.05) positive correlation with MDA and Total Cholesterol (TC). Hcy shows no significant (p>0.05) positive correlation with very low density lipoprotein (VLDL), low density lipoprotein (LDL), and Triglyceride(TG). Hcy shows negative significant (p<0.05) correlation with high density lipoprotein (HDL). While in group2 Hcy shows significant (P<0.05) positive correlation with MDA, TC, VLDL, LDL and TG. Hcy shows negative significant (p<0.05) correlation with HDL. Hcy, MDA, TC, TG, LDL and VLDL were higher without significant in CAD diabetic patients than non-diabetic CAD patients. HDL was lower in diabetic coronary artery disease patients than non-diabetic coronary artery disease patients but without significant importance.

Conclusion: In this study Hcy and MDA levels obtained were found to be positively correlated with dyslipidemia in patients in both groups. Hcy may be one of the cause for development and progress of macro- and microvascular disease. Hcy and dyslipidemia were higher in CAD with DM than CAD without DM this shows that hyperglycemia may be is another factor to increase atherosclerotic process.

Keywords: Diabetes mellitus, Homocysteine, Malondialdehyde, Dyslipidemia.

Introduction

Diabetes mellitus (DM) is an epidemiological disease widely prevalent throughout the world. DM is a significant cause of mortality and morbidity worldwide because of its serious complications such as micro- and macroangiopathic complications[1,2]. Globally studies shows about 300 million people will suffering from diabetes in 2025[1]. One of diabetic complication is coronary artery disease or cardiovascular diseases (CAD or CVD). CAD is the commonest cause of heart disease and the most important cause of death in the different countries of worldwide. Beside the known classical risk factors of CAD like hypertension, cigarette smoking, diabetes mellitus, low HDL, obesity and reduce physical inactivity, a few new parameters at last few years have been identified and studied as risk factors.
factors for development and progress of the CAD such as prothrombotic factor, pro-inflammatory factor and homocysteine\cite{3}. Homocysteine is sulfur containing amino acid formed as intermediate product in normal biosynthesis of methionine. In human body there are two major pathways to metabolize homocysteine, both of these pathways required vitamin B6, B12 and folic acid \cite{4}. One of the ways by which homocysteine increases the damage to the cardiovascular system is the formation of reactive oxygen species (ROS) by auto oxidation of homocysteine. The ROS produces by auto-oxidation of homocysteine including hydrogen peroxide and superoxide which enhances oxidative stress by production free radicals which causes lipid peroxidation to formation aldehydes. The most commonly measured compound produced by action of free radical is malondialdehyde (MDA)\cite{5}. The results of many clinical and epidemiological studies have been showed a positive correlation between pathogenesis of CAD and increasing homocysteine levels. Few studies have been carried out to established correlation between homocysteine and oxidative stress in diabetic patients with and without CAD\cite{6-10}. Therefore the study has been current to establish the correlation of hyperhomocysteinemia with oxidative stress as one of the risk factors for coronary artery disease in diabetic patients.

**Materials and Method**

The present study is conducted on 85 male subjects, 60 subjects were of coronary artery disease patients who visited Consultative Clinic of Baquba Teaching hospital in June-July 2017. The 60 CAD patients divided into two groups: group1 = 30 coronary artery disease patients without diabetic and group2 = 30 diabetic patients with coronary artery disease. The patients were compared with 25 healthy subjects were chosen as control group. All samples were collected in the morning after overnight (8-12 hours) of fasting. 6 ml of vein blood was drawn by venipuncture using a 10 ml disposable syringes from each subjects. 3ml of blood transferred into an EDTA containing vacutainer tube for determination of plasma MDA and homocysteine, and 3 ml of blood was transferred into a plain tube for evaluation of lipid profile. Estimation of plasma homocysteine was done by competitive Elisa test using commercially available kit. Estimation of serum TC, HDL-C and TG were done by using commercially available kits. Serum LDL-C and VLDL-C were calculated by using Friedewald’s formula. Estimation of plasma MDA malondialdehyde was done by colorimetric method.

Statistical analysis were done for tabulated results of all undertaken parameters by the Pearson correlation and t-test from which p value were obtained.

**Results**

Table 1 below represents the Mean±SD values for different parameters in group 1 as compared with control group. The patients of group1 and healthy controls were matched in age and body mass index(BMI), but fasting blood glucose was higher non-significantly in patients of group1 than control group. Plasma level of Hcy and MDA were significantly higher (p<0.05) in group 1 than controls. Levels of TC, TG, LDL-C and VLDL-C were higher in group1 than in controls, whereas level of HDL-C was lower in the group1 in comparison to controls. The difference is statistically significant only for TC, VLDL-C and HDL-C. Hcy showed significant positive correlation with TC, and significant negative correlation with HDL-C as shown in table 2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD of control (n=25)</th>
<th>Mean ± SD of group1 (n=30)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48.34±12.23</td>
<td>51.65±13.56</td>
<td>0.34</td>
</tr>
<tr>
<td>BMI (Kg/m2)</td>
<td>24.6 ± 5.32</td>
<td>26.65 ± 4.98</td>
<td>0.42</td>
</tr>
<tr>
<td>FBS (mg/dl)</td>
<td>95.67±12.33</td>
<td>107.78±54.91</td>
<td>0.101</td>
</tr>
<tr>
<td>Hcy μmol/L</td>
<td>13.44±.9488</td>
<td>25.6917 ± 1.789</td>
<td>0.001</td>
</tr>
<tr>
<td>MDA μmol/L</td>
<td>0.517 ± 0.0449</td>
<td>1.183 ±0.1108</td>
<td>0.001</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>172.30 ± 6.60</td>
<td>189.62 ± 8.42</td>
<td>0.02</td>
</tr>
</tbody>
</table>
Table 1: Shows the comparison of M±SD for parameters of group1 with control group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD of control (n=25)</th>
<th>Mean ± SD of group1(n=30)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(years)</td>
<td>48.34±12.23</td>
<td>53.44±14</td>
<td>0.23</td>
</tr>
<tr>
<td>BMI(Kg/m2)</td>
<td>24.6 ± 5.32</td>
<td>25.11±7.21</td>
<td>0.44</td>
</tr>
<tr>
<td>FBG(mg/dl)</td>
<td>95.67±12.33</td>
<td>196.34±98.66</td>
<td>0.000</td>
</tr>
<tr>
<td>Hcy µmol/L</td>
<td>13.4467 ± .9488</td>
<td>27.971 ± 1.789</td>
<td>0.001</td>
</tr>
<tr>
<td>MDA µmol/L</td>
<td>0.517 ± 0.0449</td>
<td>1.253 ±0.181</td>
<td>0.000</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>122.27± 7.40</td>
<td>150.05 ± 8.35</td>
<td>0.053</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>107.30 ± 6.19</td>
<td>120.67 ± 7.17</td>
<td>0.197</td>
</tr>
<tr>
<td>VLDL-C (mg/dl)</td>
<td>23.5 ± 1.42</td>
<td>30.95 ± 2.36</td>
<td>0.036</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>42.03 ± 1.36</td>
<td>37.35 ± 1.33</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Table 2: shows the correlations between parameters of group1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TG</th>
<th>TC</th>
<th>LDL</th>
<th>VLDL</th>
<th>HDL</th>
<th>MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hcy</td>
<td>0.190</td>
<td>0.333*</td>
<td>0.121</td>
<td>0.211</td>
<td>-0.288*</td>
<td>0.617*</td>
</tr>
<tr>
<td>MDA</td>
<td>0.157</td>
<td>0.403*</td>
<td>0.203</td>
<td>0.225</td>
<td>-0.182</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 3 below also represents the Mean±SD values obtained for different parameters in group2 in comparison with control group. As in group1 patients of group2 and healthy controls were matched in age and body mass index(BMI), but fasting blood glucose was normally, significantly higher in diabetic patients of group2 than control group. Hcy and MDA levels were significantly higher (p<0.05) in group2 than controls. The TC, TG, LDL-C and VLDL-C levels also, were higher in group2 than in controls, whereas level of HDL-C was lower in the group2 in comparison to controls. The difference is significant statistically only for TC, TG, VLDL-C and HDL-C. Hcy showed significant positive correlation with TC, LDL-C,VLDL-C and TG and negative significant correlation with HDL-C as shown in table4.

Table 3: Shows the comparison of M±SD for parameters of group2 with control group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD of control (n=25)</th>
<th>Mean ± SD of group1(n=30)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(years)</td>
<td>48.34±12.23</td>
<td>53.44±14</td>
<td>0.23</td>
</tr>
<tr>
<td>BMI(Kg/m2)</td>
<td>24.6 ± 5.32</td>
<td>25.11±7.21</td>
<td>0.44</td>
</tr>
<tr>
<td>FBG(mg/dl)</td>
<td>95.67±12.33</td>
<td>196.34±98.66</td>
<td>0.000</td>
</tr>
<tr>
<td>Hcy µmol/L</td>
<td>13.4467 ± .9488</td>
<td>27.971 ± 1.789</td>
<td>0.001</td>
</tr>
<tr>
<td>MDA µmol/L</td>
<td>0.517 ± 0.0449</td>
<td>1.253 ±0.181</td>
<td>0.000</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>122.27± 7.40</td>
<td>150.05 ± 8.35</td>
<td>0.053</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>172.30 ± 6.60</td>
<td>208.96 ± 30.22</td>
<td>0.019</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>107.30 ± 6.19</td>
<td>132.77 ± 9.37</td>
<td>0.097</td>
</tr>
<tr>
<td>VLDL-C (mg/dl)</td>
<td>23.5 ± 1.42</td>
<td>35.90 ± 3. 26</td>
<td>0.028</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>42.03 ± 1.36</td>
<td>33.35 ± 1.33</td>
<td>0.021</td>
</tr>
</tbody>
</table>
Table 4: shows the correlations between parameters of group2.

<table>
<thead>
<tr>
<th>parameter</th>
<th>TG</th>
<th>TC</th>
<th>LDL</th>
<th>VLDL</th>
<th>HDL</th>
<th>MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hcy</td>
<td>0.390*</td>
<td>0.441*</td>
<td>0.321*</td>
<td>0.411*</td>
<td>-0.328*</td>
<td>0.617*</td>
</tr>
<tr>
<td>MDA</td>
<td>0.357*</td>
<td>0.413*</td>
<td>0.204</td>
<td>0.275*</td>
<td>-0.182</td>
<td>1</td>
</tr>
</tbody>
</table>

Generally, all levels of studied parameters showed increasing in group2 (diabetic patients with CAD) in comparison to group1 (CAD without diabetic patients) but the differences do not showed statistical significant (p>0.05) as shown in table 5.

Table 5: shows the comparison between group1 and group2

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean ± SD of group1(n=30)</th>
<th>Mean ± SD of group2(n=30)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBG(mg/dl)</td>
<td>107.78±54.91</td>
<td>196.34±98.66</td>
<td>0.15</td>
</tr>
<tr>
<td>Hcy µmol/L</td>
<td>25.6917 ± 1.789</td>
<td>27.971 ± 1.789</td>
<td>0.27</td>
</tr>
<tr>
<td>MDA µmol/L</td>
<td>1.183 ±0.1108</td>
<td>1.253 ±0.181</td>
<td>0.21</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>189.62 ± 8.42</td>
<td>208.96 ± 30.22</td>
<td>0.13</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>150.05 ± 8.35</td>
<td>175.15 ± 12.53</td>
<td>0.09</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>120.67 ± 7.17</td>
<td>132.77 ± 9.37</td>
<td>0.16</td>
</tr>
<tr>
<td>VLDL-C (mg/dl)</td>
<td>30.95 ± 2.36</td>
<td>35.90 ± 3.26</td>
<td>0.19</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>37.35 ± 1.33</td>
<td>33.35 ± 1.33</td>
<td>0.34</td>
</tr>
</tbody>
</table>

Discussion

The statistical positive significant correlation between Hcy and MDA levels that obtained in study is agreed with some previous studies[6-11] that shows positive correlation between Hcy and MDA levels (p<0.05). The exact mechanism of toxicity by Hcy is still unknown well, but it’s believed that homocysteine or compounds liberated from its metabolize enhancing atherosclerosis by several mechanisms for example the hydrogen peroxide generated by auto oxidation of homocysteine which affect toxically on endothelial cells[11-13]. Generated hydrogen peroxide promotes oxidation of low density lipoproteins(LDL) and lipids peroxidation. The oxidized LDL causes alteration of metabolism for nitric oxide (NO) the oxidative damages also causes alteration of functions on vascular endothelial cells and platelets[5]. Auto-oxidation of homocysteine beside generation of hydrogen peroxide, it is also generates superoxide anion and hydroxyl radical, and all these compounds are factors contributing for vascular injury that is associated with hyperhomocysteinemia[11-14]. Also the auto-oxidation of Homocysteine to produce the free radicals which are the initiators of lipid peroxidation in cells. Lipid peroxidation induced by homocysteine is more occurrence in hyperlipidemic state which can increases the production of MDA through enhance oxidative stress[11-14]. As shown in table 3 diabetes mellitus increases risk factors levels of CAD. Although, the possible role of hyper Hcy in diabetes is not clear. Hyperglycemia may another risk factor with HHcy increases the oxidative stress and contributes to acceleration the atherosclerotic process in diabetes mellitus[15]. So, HHcy may accelerate vascular damage related to diabetes. Some studies re-searching the relationship between plasma Hcy and diabetes have shown that type 2 diabetic patients have a higher prevalence of HHcy than control subjects[16,17].

Conclusion

Hcy and MDA levels obtained in this study were found to be positively correlated with each other and with lipid parameters in CAD patients without diabetic and diabetic patients with CAD but the increase were higher in diabetic patients with CAD than CAD patients whom without diabetic. This indicates that Hcy enhances
the oxidative stress by lipid peroxidation, HHcy may accelerate vascular damage related to diabetes this may be one of the cause for development of coronary artery disease in diabetes rapidly, and, Hyperglycemia may another risk factor with HHcy increases the oxidative stress and contributes to acceleration the atherosclerotic process in diabetes mellitus.

**Ethical Clearance:** This study was conducted with the consent of the volunteers and without mentioning the names with the complete privacy of volunteers

**Source of Funding:** Self

**Conflict of Interest:** There were no Conflicts of Interest: in this study.

**References**


Determination of Some Biomarkers that affect in Behaviors of Autism Spectrum Disorder Individuals in Iraq

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¹Department of Chemistry, College of Science, Basra University, Iraq

Abstract

Autism is a developmental disorder characterized by troubles with social interaction and communication by restricted repetitive behavior. The parents usually notice signs in the first two or three years of their child’s life. These signs often develop gradually. Blood samples were taken for 60 people with autism and 30 healthy people as a control group. Both groups ranged age from 3 to 8 years. After completion of number, required tests were carried out on samples. The tests were three biochemical markers. The first is neurotransmitters (γABA), the other enzymes of happiness (Serotonin & Melatonin), all this biochemical markers were measured by elisa technique, the results were a decrease in level of γABA for ASD as compare with control group, A significant increases in serotonin in ASD relative to control group, and no change in level of Melatonin in both ASD and healthy group these results consistent with many research and another research inconsistent with these results have shown the reasons that led to differences in results.

Key word: Autism, GABA, Serotonin & Melatonin

Introduction

“Autism is a developmental disorder characterized by troubles with social interaction and communication by restricted repetitive behavior. Parents usually notice signs in the first two or three years of their child’s life. These signs often develop gradually, though some children with autism reach their developmental milestones at a normal pace and then worsen” ¹ ² so researchers were characterized the “Autism spectrum disorders” by vast lack of social communication skills and skill of attraction or care between the patient and society. According to recent studies carried out at one of the American centers that deal with diseases and methods of prevention ³, a statistical study showed (ASD) affects (1) child from (68) less than eight years old, ⁴ the great challenges facing developmental neuroscience is to understand how complex social interactions can shape brain development during ontogeny to affect adult functioning in social and behavioral contexts. Additionally the type of autism Spectrum Disorders (ASDs), including Autistic Disorder (AD), Asperger’s Syndrome as Childhood disintegrative disorder (CDD), Rett’s Syndrome (RS) and Pervasive Developmental Disorder Not Otherwise Specified (PDD-NOS) represent a cluster of instability in the growth of nervous which suffers from this cluster as a result is the inability to social communication at all levels, the excessive behavior repeated since the third year age ⁵.

So research was connect between (ASD) and neurotransmitter when they explain gamma-Aminobutyric acid, γ-aminobutyric acid, GABA which is the chief inhibitory neurotransmitter in the developmentally mature mammalian central nervous system. Its principal role is reducing neuronal excitability throughout the nervous system. In humans, GABA is also directly responsible for the regulation of muscle tone ⁶.

One of the factors that has revolved around the information on autism and Serotonin level in autistic children and assess the reliability of serum serotonin levels as a biomarker for diagnosis of ASD. Serotonin influences many physiological activities in the human body ⁷.

Another studies that deals with Melatonin and its relative with (ASD) This hormone is primarily produced by the pineal gland, several factors have been shown to alter circadian rhythms and melatonin levels, abnormal melatonin secretion has been implicated in circadian disturbances and neurodevelopmental abnormalities including autism spectrum disorder (ASD) ⁸.
Subjects & Method

1- Subjects

1-1- Patients: 60 Iraqi children with Autism spectrum disorder (ASD) and 30 apparently healthy Adult were selected as the control group. Their ages ranged between 3-8 years. These children (ASD) were registered in many specialized centers that care with autism in Iraq particularly in the governorates of Basra, Karbala, Babylon, Baghdad and Najaf. Where the blood sample was taken from them after the approval of their parents. The present study excluded the children (ASD) from apparent other mental disease.

1-2- Blood Samples

A Five milliliters of venous blood samples were collected from each children of (ASD) and control subject. then serum was separated and transported into new disposable tubes and freezes at (-70 °C) until assayed.

2- Methods: Measuring each of (Gama amino butyric acid (γABA), Serotonin and Melatonin) markers by ELISA ElabSciences kit.

Statistical analysis: The data collated after biochemical analysis were subjected to statistical calculation using statistical software (Megastat). The mean, standard deviation of mean, F-distribution test were obtained. Critical value or test of probability less than 0.05 (p< 0.05) was regarded significant also we use Microsoft Excel (2010), SPSS 17, and Minitab v. 14.

Results and Discussion

This study involved 60 patients with autism (42 males and 18 females) and 28 controls (17 males and 11 female).
The statistical analysis was divided into two sections, so the first was divided into three parts: the first was dealing with all samples, the second was concerned with male samples, and finally, the third part dealt with female samples. The second section dealt with the relationship between all items, which was measured in patient samples only.

Table (1) level of parameters (γABA, Serotonin, & Melatonin) for patients with ASD as comparison as control group.

<table>
<thead>
<tr>
<th>Items</th>
<th>Patients = 60</th>
<th>Control = 28</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>γABA</td>
<td>275.3±101.7</td>
<td>371.9±173.7</td>
<td>0.009685</td>
</tr>
<tr>
<td>Serotonin</td>
<td>160.7±75.6</td>
<td>124.8±73.9</td>
<td>0.04031</td>
</tr>
<tr>
<td>Melatonin</td>
<td>121.1±52.7</td>
<td>149.7±68.2</td>
<td>0.05623</td>
</tr>
</tbody>
</table>
The table (1) shows a high significant decrease (p value = 0.0097) in level of γABA for the case of ASD (275.3) as compare with the control group (371.9), In this paper, study γABA, serotonin and melatonin hormones. The result in table (1) shows a high significant decrease in level of γABA for the case of ASD as compare with the control group, as well the results in table (2&3) were congruous with the results of the table (1) which were a significant decrease in level of γABA as compare with their healthy group, many papers were agreement with these results, a significant reduction in the level of γABA in people with autism, the level of γABA was very low in people with autism, in addition scientists were demonstrated with all confidence that the association of autism has an inverse relationship with γABA, where the lower the level of γABA lead to increase the emergence of symptoms of autism. In a second demonstration, so the γABA level was low in those with autism at the beginning of the diagnosis.

On the other hand, there are other studies that contradict the results of current study, the research about persons suffering from autism were different groups in the ages, that the level of γABA was within the normal level, the discrepancy between current paper and the researchers’ It may be due to differences in the age of the studied cases for both studies. The age of the new research is 3-8 years, while the research whose results are opposed was about 12-16 years old. A rise in the level of γABA the attribution of reason for contrast ages of autism individuals, they said the study of autism must be for all age groups, so, the cause of different levels of γABA was attribute gap ages in people with ASD.

In contrast to γABA the level of serotonin in the table (1) shows a significant elevation (p value = 0.04) in case of ASD (160.7) as compare with their control group (124.8). Several previous studies have demonstrated a common phenomena in persons with autism is the high level of serotonin, so in this current study as shown as in table (1) a significant elevation for level of serotonin in case of ASD as compare with their control group, In a research paper on autism, Levitt P.(2011) explained the high level of serotonin in autism patients, in addition researcher was concluded that the level of serotonin increases in people with autism, so the reason for increasing the concentration of serotonin comes from a decrease in turnover of serotonin in the body and to support the results conducted experiments on a type of mice treated mice with a substance that increases the level of serotonin and found that The rate of rotation is inversely proportional to the level of serotonin in the body.

A high level of serotonin in persons with autism is one of the important signs that a good candidates for the determination of the susceptibility to autism. Many of the studies are in line with the results of the new research, serotonin plays an important role in their study of autistic patients, in the other hand the researchers found that serotonin levels were low in serum while there was a significant increase in the level of serotonin in the whole blood or platelet-rich plasma, Therefore, this study is contrary to the results found in the current study. To know why the difference in results between the current study and the opposite study is that platelets is a place for the accumulation of serotonin in its, which leads to a decrease in level of serotonin. This is what explained in their research, the relationship between the level of serotonin in people with autism in the serum and blood platelets. They also conducted a genetic study of serotonin generators.

In this study they Clarify the relationship between levels of serotonin in platelets and serum, The difference may be due to the severity of case for subjects in the study, as there is a study by Amjad where the division of cases according to severity to three classes, the serotonin levels directly proportional to the increased severity of the disease, in addition scientists were proved there is no association between low levels of serotonin and autism, while the same table (1) displays no change in level of melatonin in both ASD sample (121.1) and its control group (149.7), The result in this current research shows no significant change in level of Melatonin between autism samples and its healthy group, perhaps most of the research that deals with the study of melatonin in people of autism, shed light on the relationship of this hormone with serotonin hormone, It is a daily synchronous hormone derived from serotonin, So serotonin hormone is inversely proportional to melatonin hormone This is dependable with the results in the current search, the relationship between serotonin and melatonin is negative and the level of melatonin is low while the level of serotonin is high in people with autism compared to control group.

The level of melatonin in people with autism should be low compared with the healthy people and the time when the blood sample was drawn. They showed that melatonin levels may give abnormal levels to people with autism or may have insignificant results.
compared to Group healthy, where they attributed the abnormal results to many reasons including the situation of the ASD in a dark room or a room with a dim light, where the relationship of light with melatonin was an inverse relationship, so the lower light increased the level of melatonin in the other hand, The rise in levels of melatonin in the autistic may be caused by the use of monotherapy drugs containing a proportion of melatonin for giving them more sleep hours, leading to a higher level of autistic compared to the standard group, this search also corresponds to the current search, additionally in their research that supplements and some drugs that increase the level of melatonin in the blood of the autistic, where they conducted a search on a group of autistic people taking drugs containing melatonin and found that the level of melatonin does not differ significantly from the control group 22, these results was agreement with the current search.

### Table (2) Relationship between items for persons who are suffer from ASD.

<table>
<thead>
<tr>
<th></th>
<th>Melatonin</th>
<th>Serotonin</th>
</tr>
</thead>
<tbody>
<tr>
<td>γABA</td>
<td>0.647</td>
<td>-0.292</td>
</tr>
<tr>
<td></td>
<td>0.000</td>
<td>0.024</td>
</tr>
<tr>
<td>Serotonin</td>
<td>-0.306</td>
<td>0.017</td>
</tr>
</tbody>
</table>

The second section of biostatistical analysis which was deal about the relative among items that appear in table no. (2), so the table illustrates a highly positive significant relationship between γABA and Melatonin in persons have ASD(r = 0.647 & P value = 0.000), while in the same table demonstrates a negative significant relationship between γABA and Serotonin in same cases of ASD (r = -0.292 & P value = 0.024), additionally the table appears a negative significant relationship between Serotonin and Melatonin with individuals who suffer from ASD (r = -0.306 & P value = 0.017).

In recent years concern has risen about the increasing prevalence of developmental disorders. A striking example is Autism Spectrum Disorders (ASD). In this study which is interested in studying some of the biochemical factors and their effect on individuals who suffer from autism, neurodevelopmental disorders primarily diagnosed in childhood.

Many researchs that agree with the recent study as the individual of ASD suffers from a lack of the level of γABA, at the same time also suffers from a deficiency in the level of melatonin, since melatonin is composed of serotonin, which means that the higher the level of melatonin decreased the level of serotonin and vice versa, This is agreed upon by 23 and 24.

**Ethical Clearance:** Authors’ Contribution; All authors have made substantial, direct and intellectual contribution to the work and approved it for publication.

**Ethics Statement:** This article contain studies with human participants performed by the authors, the approval of collecting samples from the parents of the persons of the donors.

**Fundig:** None.

**Conflicts of Interest:** The authors declare that there is no conflicts of interest.

**Data availability:** All datasets generated or analyzed during this study are included in the manuscript.

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25- Basim M. Ali. “Study Effect of Blood Glucose Level on Renal Failure in Peoples Suffering from Diabetes” 5:(2016)
Detection of VT1 and VT2 genes in *Escherichia coli* isolated from Diarrhea Patients in AL-Anbar, Iraq using PCR Sequencing

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Abstract

Enteric-borne diarrheal illnesses are significant causes of human morbidity and mortality. Over 2 million diarrhea-related fatalities happen annually, especially among kids under the age of five. During the period extended from February 2019 to June 2019, A total of 196 stool locally specimens from diarrheal patients samples were collected from AL-Anbar city of AL-Ramadi hospital and AL-Fallujah hospital out of 196 samples diagnosis by biochemical test, morphology and selective chromogenic medium, found 80 samples of E. coli. The aim of the study detection of VT1 and VT2 genes in Escherichia coli isolated from Diarrhea patients by using PCR Sequencing. The result showed when diagnosis by PCR for Sanger Sequencing technique for VT1 and VT2 genes E. coli O157:H7, and E. coli O25b:H4 were positive for VT1 gene, and Escherichia coli O25b:H4, and Escherichia coli O18:H1 positive for VT2 gene.

Key words: *Escherichia coli*, diarrhea patients, VT1 and VT2 genes.

Introduction

Diarrheal illnesses are a significant cause of low-to-middle-income morbidity and mortality nations and are predestined to be the second most important reason from death among kids < 5 years of age, resulting in 0.5 million fatalities worldwide¹. Sub-Saharan and South-East Asian areas have the largest illness burden (> 72%). In spite of the known actuality that diarrheal illnesses are transferred by stool oral route (²). It is a complicated syndrome because it includes interaction of environmental, dietary and infectious variables (³). *Escherichia coli* is extremely multilateral bacterium that develops its commensal and pathogenic capacity in human host. Diarrheagenic E. coli (DEC) is recorded as one of the world’s major causes of gastrointestinal turmoil and is an significant public health issue⁴⁻⁷. These pathotypes likewise play a significant part in the morbidity of diarrhea in the Indian people. Remarkably, distinguish DEC pathotypes offer particular, virulence arsenal which convert the prevalent repertoire available for diagnostic and therapeutic methods. DEC is moreover cataloged into different pathotypes based upon appearance from these virulence determinants participating to specific pathophysiology⁸. Human and bovine STEC strains detailed two powerful phage-encoded cytotoxins called Shiga toxins (Stx1 and Stx2) or Verotoxin (VT1 and VT2)⁹⁻¹⁰. Moreover, Shiga-like toxin (Stx)-producing Escherichia coli (STEC) contagion is an continuing health issue that can leadership to severe complications, inclusive hemolytic uremic syndrome (HUS) and death¹¹.

Materials and Method

Subjects and specimen collection.

A total of 196 stool samples of patients with diarrhea aged among (2-60) yearsthrough the period from February 2019 to June 2019. Samples were collected from AL-Anbar city of AL-Ramadi hospital and AL-Fallujah hospital out of 196 samples diagnosis by biochemical test, morphology and selective chromogenic medium, found 80 samples of E. coli. Patients with soft stool as a major complications, furthermore reported another clinical appearance likewise dehydration, vomiting, Fever, common abdominal pain and mucus. Patients have been given written consent. To identify *E.coli*, stool specimens were plated into Differential media Sorbitol- MacConkey agar(SMAC), Chromo agar *E.coli* and Eosin methylene blue agar(EMB) Samples were cultured on differential agar and incubated aerobically at 37 C for 24-48 hours to isolate *E.coli*¹².
Extraction of total DNA

Genomic DNA of 80 Escherichia coli isolate was extracted by utilizing (DNA mini kit that was provided by G-spin DNA extraction kit, Korea) as instructed by the manufacturer’s instructions. Primers were acquired from the IDT corporation in this study.

Detection of Verotoxin (VT1 and VT2) genes by PCR

The genes of Verotoxin (VT1 and VT2) were amplified by F (5'-CGC TGA ATG TCA TTC GCT CTG C -3’) and R (5'-CGT GGT ATA GCT ACT GTC ACC -3’), sense F (5'-CTT CGG TAT CCT ATT ACC GG -3’) ,anda anti sense R (5'-CTG CTG TGA CAG TGA CAA AAC GC -3’) respectively (11). The PCR reaction mixture contains 5 μl of pre Master Mix, 1.5μl DNA, 1 μl of each forward and reverse primers, then the volume completed to 25 μl by deionized water. Thermo cycling conditions were as follows: initial denaturation at 5 min at 95 ° C, followed by 35 denaturation cycles at 95 ° C for 45 seconds, annealing at 58 ° C for 45 seconds, extension at 72 ° C for 45 seconds and final extension at 72 ° C for 7 minutes. A 70 volt/65 Amp current leaves the gel to run for 60 min. Visualization was performed with a UV transilluminator after electrophoresis. The sequencing of Verotoxin (VT1 and VT2) genes were performed at Macrogen company utilizing their ABI 3730xl genetic analyzer (Applied Biosystems, US). Online at the National Center for Biotechnology Information (NCBI) at (http://www.ncbi.nlm.nih.gov) and BioEdit, homology search was conducted utilizing the Basic Local Alignment Search Tool (BLAST) program. The outcomes were compared with information accessible online at the NCBI from the ExPASY program released by Gene Bank.

Results and Discussion

This study includes detection from the prevalence of VT1 and VT2 genes in collected samples locally from stool specimens after isolation on selective chromogenic medium and diagnosis by PCR for Sanger Sequencing technique found 1 out of 80 test samples were positive for E. coli O157:H7, 31 isolate E. coli O126:H20, 19 isolate E. coli O25b:H4, and 29 isolate for E. coli O18:H1.

In developing nations, toxigenic Escherichia coli has been acknowledged as the leading cause of diarrhea in both humans and animals (13). Such divergent pathogroups are often allocated using distinct terms such as “hybrid” “mixed virulence profiles” and “mixture of virulence” (14,15,16). Virulence genes in E. coli strains were connected with the coexisting STEC. In Germany, the United States and Slovakia, coli strains of human, animal and environmental origin have been recorded (17,18,19). Some of them related to human illness (19). Previous studies in Finland recognized hybrid STEC strains from patients and livestock. (20) Nyholm performed a comparative genomics and characterization study of such strains to determine their phylogenetic position in E. Coli and the genes they harbor to define virulence (21).

The polymerase chain reaction diagnostic techniques is rapid, easy, inexpensive protocol becoming the most commonly utilized of all molecular genetics ways for detecting important toxin genes and identifying the bacteria (22). Its elevated sensitivity, specificity methods for detect Specific sequence of nucleic acids discovered in the genome of pathogens (23). PCR products of 302bp for VT1 gene, 850bp for VT2 gene were detected in the positive and PCR product was not seen in the negative samples as seen in figure (1,2).

![Figure (1): Agarose gel electrophoresis for VT1 gene (302bp). Bands were fractionated by electrophoresis on a 1.5% agarose gel (2 h., 5V/cm, 1X TBE) and visualized under U.V. light after staining with red stain. Lane: M (M: 100bp ladder), Lane: 1,9,11,12,13 (negative PCR product).](image-url)
Figure (2):- Agarose gel electrophoresis for VT2 gene (850bp). Bands were fractionated by electrophoresis on a 1.5% agarose gel (2 h., 5V/cm, 1X TBE) and visualized under U.V. light after staining with red stain. Lane: 1 (M: 100bp ladder).

The VT1 and VT2 genes were amplified by PCR method, and sent for sequencing service to Macrogen company Korea. The sequencing result of VT1gene shows for strain Escherichia coli O25b:H4 having one Transition T>C in location (1273120 nucleotide) code TTG>CTG of amino acid Leucine>Leucine and Predicted effect nonsense , also three Transversion one in location G>C (1273126 nucleotide) code GAA>CAA amino acid change Glutamic acid to Glutamine the effect Missense , and two T>G in location (1273131 and 1273148 nucleotide respectively code GGT>GGG of the same amino acid Glycine > Glycine, that nonsense substitution , also code CTG to CGG transformation Leucine to Arginine that effect Missense. From the Gene Bank, found that part of VT1 gene having 99% compatibility with subject of VT1 gene in NCBI as seen in table (1). (24).

Table (1): Represent type of polymorphism of VT1 gene.

<table>
<thead>
<tr>
<th>Type of substitution</th>
<th>Location</th>
<th>Nucleotide</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Predicted effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transition</td>
<td>1273120</td>
<td>T&gt;C</td>
<td>TTG&gt;CTG</td>
<td>Leucine&gt;Leucine</td>
<td>nonsense</td>
</tr>
<tr>
<td>Transversion</td>
<td>1273126</td>
<td>G&gt;C</td>
<td>GAA&gt;CAA</td>
<td>Glutamic acid&gt;Glutamine</td>
<td>Missense</td>
</tr>
<tr>
<td>Transversion</td>
<td>1273131</td>
<td>T&gt;G</td>
<td>GGT&gt;GGG</td>
<td>Glycine &gt; Glycine</td>
<td>nonsense</td>
</tr>
<tr>
<td>Transversion</td>
<td>1273148</td>
<td>T&gt;G</td>
<td>CTG&gt;CGG</td>
<td>Leucine&gt;Arginine</td>
<td>Missense</td>
</tr>
</tbody>
</table>

While the analysis of the VT1 gene for strain Escherichia coli O25b:H4 our study was coordinated by 99% having one Transversion of Pyrimidine nucleotide C to Purine of nucleotide G, code ACG > AGG and amino acid transformation Threonine>Arginine the effect Missense in location (1273112 nucleotide), under sequence ID: CP015085.1, as shown in table(1) \(^{(24)}\).

Compatibility of 97 percent in Gene Bank of VT1 gene as shown in table (4) under sequence ID: CP015085.1 having one Transition A to G in location (1273045 nucleotide), and code ATC>GTC amino acid Isoleucine>Valine and Missense substitution , as well three Transversion A to T in location (1273037 nucleotide), the code AAT>ATT amino acid change Asparagine to Isoleucine , another location C>G (1273049 nucleotide), have code GCC>GGC and amino acid Alanine> Glycine.
having eight Transition showed three C to T in sites (1478626, 1478533, and 1478368 nucleotide) having a code and not changing amino acid ACC>ACT Threonine> Threonine, TCC>TCT Serine> Serine, and CCC>CCT Proline>Proline they have no impact nonsense, also one transition T to C in site (1478620 nucleotide) the code TTT>TTC, and then the same amino acid Phenylalanine >Phenylalanine, as well as three Transition G to A in sites (1478554, 1478293, and 1478224 nucleotide), having the code CAG>CAA, GCG>GCA, and CAG>CAA, the acids of the amino respectively Glutamine to Glutamine, Alanine to Alanine, and Glutamine to Glutamine that nonsense substitution, While A > G is in place (1478416 nucleotide), the code CAA > CAG and the amino acid Glutamine to Glutamine has an effect nonsense. Two Transversion as displaying one A > C (1478524 nucleotide), code GCA > GCC amino acid not altering Alanine > Alanine, and predicted nonsense impact, finally appeared G to T in location (1478405 nucleotide), the code AGC>ATC, and amino acid alteration Serine> Isoleucine the Predicted effect Missense. From the Gene Bank, part of the VT1 gene for strain Escherichia coli O157:H7 was found to be 98% compatible with the NCBI standard, as shown in table (2).

**Table (2): Represent type of polymorphism of VT1 gene.**

<table>
<thead>
<tr>
<th>Type of substitution</th>
<th>Location</th>
<th>Nucleotide</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Predicted effect</th>
<th>Sequence ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transversion</td>
<td>1273037</td>
<td>A&gt;T</td>
<td>AAT&gt;ATT</td>
<td>Asparagine&gt; Isoleucine</td>
<td>Missense</td>
<td>ID: CP015085.1</td>
</tr>
<tr>
<td>Transition</td>
<td>1273045</td>
<td>A&gt;G</td>
<td>ATC&gt;GTC</td>
<td>Isoleucine&gt;Valine</td>
<td>Missense</td>
<td></td>
</tr>
<tr>
<td>Transversion</td>
<td>1273049</td>
<td>C&gt;G</td>
<td>GCC&gt;GGC</td>
<td>Alanine&gt;Glycine</td>
<td>Missense</td>
<td></td>
</tr>
<tr>
<td>Transversion</td>
<td>1273135</td>
<td>C&gt;A</td>
<td>CCT&gt;ACT</td>
<td>Proline&gt;Threonine</td>
<td>Missense</td>
<td></td>
</tr>
</tbody>
</table>

**Table (3): Represent type of polymorphism of VT1 gene.**

<table>
<thead>
<tr>
<th>Type of substitution</th>
<th>Location</th>
<th>Nucleotide</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Predicted effect</th>
<th>Sequence ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transition</td>
<td>1478626</td>
<td>C&gt;T</td>
<td>ACC&gt;ACT</td>
<td>Threonine&gt;Threonine</td>
<td>nonsense</td>
<td></td>
</tr>
<tr>
<td>Transition</td>
<td>1478620</td>
<td>T&gt;C</td>
<td>TTT&gt;TTC</td>
<td>Phenylalanine&gt;Phenylalanine</td>
<td>nonsense</td>
<td></td>
</tr>
<tr>
<td>Transition</td>
<td>1478554</td>
<td>G&gt;A</td>
<td>CAG&gt;CAA</td>
<td>Glutamine&gt;Glutamine</td>
<td>nonsense</td>
<td></td>
</tr>
<tr>
<td>Transition</td>
<td>1478533</td>
<td>C&gt;T</td>
<td>TCC&gt;TCT</td>
<td>Serine&gt;Serine</td>
<td>nonsense</td>
<td></td>
</tr>
<tr>
<td>Transversion</td>
<td>1478524</td>
<td>A&gt;C</td>
<td>GCA&gt;GCC</td>
<td>Alanine&gt;Alanine</td>
<td>nonsense</td>
<td>ID: CP040572.1</td>
</tr>
<tr>
<td>Transition</td>
<td>1478416</td>
<td>A&gt;G</td>
<td>CAA&gt;CAG</td>
<td>Glutamine&gt;Glutamine</td>
<td>nonsense</td>
<td></td>
</tr>
<tr>
<td>Transversion</td>
<td>1478405</td>
<td>G&gt;T</td>
<td>AGC&gt;ATC</td>
<td>Serine&gt;Isoleucine</td>
<td>Missense</td>
<td></td>
</tr>
<tr>
<td>Transition</td>
<td>1478368</td>
<td>C&gt;T</td>
<td>CCC&gt;CCT</td>
<td>Proline&gt;Proline</td>
<td>nonsense</td>
<td></td>
</tr>
<tr>
<td>Transition</td>
<td>1478293</td>
<td>G&gt;A</td>
<td>GCG&gt;GCA</td>
<td>Alanine&gt;Alanine</td>
<td>nonsense</td>
<td></td>
</tr>
<tr>
<td>Transition</td>
<td>1478224</td>
<td>G&gt;A</td>
<td>CAG&gt;CAA</td>
<td>Glutamine&gt;Glutamine</td>
<td>nonsense</td>
<td></td>
</tr>
</tbody>
</table>
Another part of sequencing for VT2 gene to strain Escherichia coli O25b:H4 the result shows Compatibility of 100% in Gene Bank of VT2 gene for Escherichia coli O25b:H4 under sequence ID: CP015085.1, so no recorded change noticed from the Gene Bank in VT2 gene (25).

Amplification of the VT2 gene for Escherichia coli O18:H1 with one Transition T > C in intron place (1902685 nucleotide) and two Transition T to C place (1903016, and 1903166 nucleotide), code ATT > ATC, and CGT > CGC amino acids respectively Isoleucine > Isoleucine, Arginine > Arginine, and predicted nonsense impact.

The sequencing analysis of VT2 gene for Escherichia coli O18:H1 as seen in table (3). The our study was coordinated 99% having two Transversion T to A in place (1902482 nucleotide ) code TAA>AAA and amino acid alteration Leucine to Phenylalanine, another Transversion from Pyrimidine nucleotide C to Purine of nucleotide G in place (1902494 nucleotide ) that code CTT to GTT, amino acid alteration Lysine to Asparagine the effect Missense, also one Transition T to Cin intron, and two Transition T to C in locations (1903016 and 1903166 nucleotide ), code TTG>TGC , and CGT>CGC , the amino acid not altering Isoleucine> Isoleucine, and Arginine> Arginine respectively and nonsense substitution. under sequence ID: CP028320.1.

Table (4): Represent type of polymorphism of VT2 gene.

<table>
<thead>
<tr>
<th>Type of substitution</th>
<th>Location</th>
<th>Nucleotide</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Predicted effect</th>
<th>Sequence ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transversion</td>
<td>1902482</td>
<td>T&gt;A</td>
<td>TAA&gt;AAA</td>
<td>Leucine&gt; Phenylalanine</td>
<td>Missense</td>
<td></td>
</tr>
<tr>
<td>Transversion</td>
<td>1902494</td>
<td>C&gt;G</td>
<td>CTT&gt;GTT</td>
<td>Lysine&gt; Asparagine</td>
<td>Missense</td>
<td></td>
</tr>
<tr>
<td>Transition</td>
<td>1902685</td>
<td>T&gt;C</td>
<td>INTRON</td>
<td></td>
<td></td>
<td>ID: CP028320.1</td>
</tr>
<tr>
<td>Transition</td>
<td>1903016</td>
<td>T&gt;C</td>
<td>TTG&gt;TGC</td>
<td>Isoleucine&gt; Isoleucine</td>
<td>nonsense</td>
<td></td>
</tr>
<tr>
<td>Transition</td>
<td>1903166</td>
<td>T&gt;C</td>
<td>CGT&gt;CGC</td>
<td>Arginine&gt; Arginine</td>
<td>nonsense</td>
<td></td>
</tr>
</tbody>
</table>

For the first time in Iraq, this study explored VT1 and VT2 genes using Sanger Sequencing method where few research was conducted to detect those samples through sequencing. Few studies have assessed or compared easy DNA extraction techniques to enable and improve the sensitivity of fecal samples to PCR detection of enteric pathogens (26, 27).

**Conclusions**

In the present study in stool samples of patients with diarrhea when diagnosis by PCR for Sanger Sequencing technique for VT1 and VT2 genes E. coli O157:H7, and E. coli O25b:H4 were positive for VT1 gene, and Escherichia coli O25b:H4, and Escherichia coli O18:H1 positive for VT2 gene.

**Conflict of Interest:** There is no conflict of interest among the authors.

**Funding:** Self

**Ethical Clearance:** This study is ethically approved by the Institutional ethical Committee.

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Correlation between the Duration of Ethambutol Therapy and the Toxic Optic Neuropathy Occurrence in Patients with Multidrug-Resistance Tuberculosis

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Abstract

Background: Tuberculosis affects one-third of the world’s population. Increasing number of tuberculosis cases also leads to the increase of anti-tuberculosis drugs use, such as ethambutol. Ethambutol is one of the most effective and rarely resistant tuberculosis drugs.

Objective: To determine the relationship between duration of ethambutol therapy with toxic optic neuropathy in tuberculosis patients

Method: This study enrolled MDR-TB patients who visited MDR-TB Unit of Dr. Soetomo General Hospital from July 2014 until the sufficient amount of sample was achieved. The patients enrolled are those who met the inclusion and exclusion criteria. Inclusion criteria consisted of MDR TB patients treated with ethambutol therapy, aged of 20-69 years old, and whose VEP examination results showed extension of P100 latency.

Results: The mean of <6 month duration was 3.24±1.348, while for the ≥6 months was 11.71±5.764. This difference was statistically significant with p = 0.043.

Conclusion: There is a correlation between duration of ethambutol therapy with toxic optic neuropathy in patients with MDR-TB.

Keywords: ethambutol therapy, toxic optic neuropathy, multidrug-resistance, visual evoked potentials

Introduction

Tuberculosis affects one-third of the world’s population. The increasing number of tuberculosis cases leads to the more use of anti-tuberculosis drugs, such as ethambutol. Ethambutol is one of the most effective and rarely resistant tuberculosis drugs, and therefore is often used in the case of Multidrug-Resistance Tuberculosis (MDR-TB). However, many researches reported the occurrence of optical toxic neuropathy (TON) with clinical symptoms of decreased vision until blindness.

The incidence of toxic optic neuropathy associated with ethambutol varies greatly from several studies ranging from 0.5-35% of patients. Visual Evoked Potentials (VEP) can be used to determine the toxic effects of ethambutol. Of the 14 samples, 5 were showed abnormalities in VEP, where in from the clinical findings only showed abnormal person. A study reported that 42% of patients with permanent vision impairment were dependent on the initial disorder severity. As reported in most cases, toxicity occurs at doses greater than 20mg/kgBW/day. However, not too little toxicity has occurred at a standard dose of 15mg/kgBW/day. For instance is in a case reporting optic neuropathy occurrence at a dose of 12 mg/kgBW/day. The duration of drug use that causes toxic effects is averagely one to six months. However, it has been reported that the fastest duration in which
visually impaired due to ethambutol occurs in the use duration of only three days.

Toxic optic neuropathy can provide symptoms of progressive decrease in vision, which might get worse until blindness occurs. Although the loss of vision is reversible when ethambutol is lowered or terminated, some patients experience permanent vision impairment even with standard drug doses. Some cases of progressiveness continue to run even though the drug has been stopped. Imperfect improvement was often found. Some even experienced permanent blindness. The mechanism of optic neuropathy due to ethambutol is thought to be caused by impaired function of the mitochondria. One study found a vacuole change from the cytoplasm of ganglion cells in animals injected with ethambutol. Ethambutol is a metal chelator, which can interact with zinc, copper and iron that cause damage to the mitochondria. Studies conducted on animals have demonstrated the toxic effects of ethambutol on electrophysiological visual systems as well as histopathology.

Toxicity can be detected earlier by VEP, even before the emergence of complaints from patients. This is very useful because by detecting TON earlier, can be stopped or replacement of drugs before clinical symptoms appear. In addition, knowing the occurrence of TON and drug termination will inhibit the progression of the disease and thus permanent loss of vision can be prevented. Aim to determine the relationship between duration of ethambutol therapy with toxic optic neuropathy in tuberculosis patients.

Method

This is an observational analytic study. The samples are MDR-TB patients who received ethambutol therapy who came in MDR-TB Unit of Dr. Soetomo General Hospital from July 2014 until the sufficient amount of samples was achieved. They all met the inclusion criteria consisted of MDR-TB patients who received ethambutol therapy, aged of 20-69 years old, VEP examination results obtained extension of P100 latency. The exclusion criteria consisted of the subjects with diabetic retinopathy, hypertensive retinopathy, renal failure, hepatic dysfunction, history of stroke, history of intracranial infection, multiple sclerosis history, cerebral neoplasm history, eye abnormalities, and those who were unwilling to participate in the study.

The sampling method of the study was conducted according to the consecutive case (sampling from consecutive admission) until the sample size was determined. The study instrument was visual evoked potentials. The duration of ethambutol therapy was the duration (within months) of patients taking ethambutol during the MDR program, calculated from the first day of drug use until the VEP examination with short duration of <6 months, and long duration of ≥6 months.

Toxic optic neuropathy is an abnormality in vision function due to optic nerve damage caused by toxic substances diagnosed from an extension of P100 latency from the normal limit of VEP examination with a 116 ms cutoff point. TON occurred when the obtained P100 latency is >116 ms, and it is considered normal when the normal P100 latency is ≤116 ms. VEP is the electrical potential of the visual stimulus recorded from the scalp to assess the integrity of the afferent visual path. The examination was done by Cadwell Sierra Wave in Electromyography of Dr. Soetomo General Hospital. The results will be interpreted by a neurophysiologist consultant. All subjects included in the inclusion criteria and the family responsible for the patients were given an explanation of the purpose, usefulness and risk of the study, and were asked to be involved in the study. The identity and characteristics of the subjects signing the consent were recorded in the form. Data collection of research subjects was conducted through anamnesis, careful physical examination and neurology, selection of samples for the cases according to inclusion and exclusion criteria. The eligible samples and all necessary clinical data were recorded. VEP examination was performed in the EMG of Dr. Soetomo General Hospital. All the recording results were collected for further data tabulation and statistical analysis.

The data obtained from the data collection sheets were then analyzed. The correlation between duration of ethambutol therapy and optic neuropathy was calculated and analyzed using the appropriate statistical tests. This study will be proposed as an ethical feasibility study to the Research Ethics Committee of Dr. Soetomo Teaching Hospital Surabaya.
Table 1. The difference in the proportion of duration of ethambutol therapy in each group of subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>TON</th>
<th>Control</th>
<th>p</th>
<th>RO (IK 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases N (%)</td>
<td>N (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>43.29 ± 11.58</td>
<td>38.82 ± 10.06</td>
<td>0.239</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>7 (41.2%)</td>
<td>13 (76.5%)</td>
<td>0.03</td>
<td>0.215 (0.049-0.946)</td>
</tr>
<tr>
<td>Female</td>
<td>10 (58.8%)</td>
<td>4 (23.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elementary, Junior High School</td>
<td>7 (41.2%)</td>
<td>6 (35.3%)</td>
<td>0.724</td>
<td>0.779 (0.195 - 3.118)</td>
</tr>
<tr>
<td>Senior High School, College</td>
<td>10 (58.8%)</td>
<td>11 (64.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>7 (41.2%)</td>
<td>6 (35.3%)</td>
<td>0.724</td>
<td>1.283 (0.321 – 5.134)</td>
</tr>
<tr>
<td>No</td>
<td>10 (58.8%)</td>
<td>11 (64.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethambutol dose (mg/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥1200</td>
<td>7 (41.2%)</td>
<td>10 (58.8%)</td>
<td>0.303</td>
<td>0.49 (0.125 – 1.921)</td>
</tr>
<tr>
<td>800-1199</td>
<td>10 (58.8%)</td>
<td>7 (41.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethambutol therapy history (year)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤1</td>
<td>7 (41.2%)</td>
<td>6 (35.3%)</td>
<td>0.271</td>
<td>2.275 (0.518 – 9.989)</td>
</tr>
<tr>
<td>&gt;1</td>
<td>10 (58.8%)</td>
<td>11 (64.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethambutol therapy duration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥6 months</td>
<td>12 (70.6%)</td>
<td>5 (29.4%)</td>
<td>0.016</td>
<td>5.76 (1.317-25.187)</td>
</tr>
<tr>
<td>&lt;6 months</td>
<td>5 (29.4%)</td>
<td>12 (70.6%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All subjects underwent eye examination in MDR-TB and VEP units in the EMG section of Department of Neurology, Dr. Soetomo General Hospital Surabaya from July to December 2014. In this study, there were 34 subjects consisting of 17 normal subjects with normal Visual Evoked Potential (VEP) results referred to as controls and 17 subjects with VEP prolonged latency P100 results hereinafter referred to as cases. Table 1 shows the characteristics of the study subjects. In the case group there were 7 male subjects (41.2%) which were less than in control group with 13 male subjects (76.5%). There was a difference of sex proportions between each group, and this difference was statistically significant with p = 0.037. The mean of the subjects’ age in the case group was 43.29 ± 11.58 years, while in the control group was 38.82 ± 10.06 years old. However, this difference was not statistically significant with p = 0.239. in the case group, However, this difference was not statistically significant with p = 0.724. Characteristics of subjects based on dose of ethambutol ≥1200 mg / day in case group there were 10 people (58.8%) more than control group that was 7 people (41.2%). However, this difference was not statistically significant with p = 0.303. Characteristics of subjects based on history of ethambutol therapy ≤1 years showed that in the case group there were 7 people (41.2%), which was more than the control group with 4 people (23.5%). However, this difference was not statistically significant with p = 0.271.

The difference in the proportion of duration of ethambutol therapy in each group of subjects can be seen in Table 1. In the case group, there were 12 subjects (70.6%) with ethambutol therapy duration of 5 months, which is more than the control group with only 5 subjects (29.4%). Meanwhile, in group <6 months duration there were 5 subjects in case group (294%), which is less than in control group with 12 people (70.6%). This difference was statistically significant with p = 0.016. Odd ratio of...
5.76 (95% IK 1.317-25.187) was also obtained. Logistic regression of sex and duration indicated a significant correlation between duration of ethambutol therapy and TON with p value = 0.043 and an odds ratio of 4.93 (95% IK 1.053 - 23.082). Thus, subjects with duration of ethambutol therapy ≥6 months had 4.93 times higher risk of TON compared to subjects with ethambutol therapy duration of <6 months. This suggests that the study is clinically and statistically significant.

**Discussion**

The gender characteristics of a total of 34 samples 20 (58%) male and 14 (42%) female. A population-based study in Taiwan found more male with 65.8%. Similarly, in studies that linked ethambutol therapy to another TON, there were more male subjects than females 71%.

The mean age of the subject of the case group was 43.29 ± 11.58 years old while in the control group was 38.82 ± 10.06 years old, as seen in the older average age group. These demographic data were compared with studies that correlated the dose of ethambutol with TON obtained in the mean age of 55.4 ± 14.4 years old. The study of TON incident, the obtained the mean of age range was 58.23 ± 16.68 years. Characteristics of subjects based on risk factors of diabetes mellitus in case group was 7 people (41.2%) slightly more than control group 6 people (35.3%), but this difference was not statistically significant with p = 0.742.

The dose of ethambutol of ≥1200 mg/day in the case group was obtained in 10 subjects (58.8%) more than in the control group with 7 subjects (41.2%). While the dose of 800-1199 mg/day were found in case group with 7 subjects (41.2%) less than control group that was 10 subjects (58.8%), was not statistically significant. The study with 231 TON samples was 49.8% dose <800 mg, 38.1% dose 800-1199 mg, 12.1% dose ≥1200 mg/day (3). Based on the history of ethambutol therapy of ≤1 years, in case group there were 10 subjects (41.2%), which is more than control group with 4 subjects (23.5%). However, this difference was not statistically significant with p = 0.271.

The previous studies have not reported about the history of the use of ethambutol before, possibly because the previous studies involved a sample of TB patients category 1, whereas in this study the samples are MDR-TB patients who had previously received ethambutol therapy. In the case group, the number of subjects with ethambutol therapy duration of ≥6 months was 12 (70.6%), more than in control group with 5 subjects (29.4%). Meanwhile in the control group, the number of subjects with ethambutol therapy duration of <6 months is 12 (70.6%) which is more than in the case group with 5 people (29.4%). The mean of <6-month-duration was 3.24 ± 1.348, while for the ≥6-month-duration was 11.71 ± 5.764. This difference is significant statistically with p = 0.043, and clinically with odds of 4.93 (95% 95% 95%-23.082).

This study was conducted using the cut-off of >3 months. In case group, there were 143 subjects (61.9%) with 3-month duration and 88 subjects (38.1%) with ≤3-month duration, with odds ration of 1.35 (1.02-1.86). The case series performed by Griffith et al. found that the duration of ethambutol in the case group was 1-16 months (6.7 ± 5.8 months), with a tendency for longer duration using ethambutol with optical neuropathy.

Long duration of ethambutol therapy might cause TON. This can be analyzed from the following explanation: ethambutol is a metal chelator, this agent is able to strongly bind metals especially zinc required in axonal transport, and citric acid cycle. Ethambutol bonding to these metals results in calcium influx, mitochondrial dysfunction, and ultimately leads to decreased ATP, ganglion cell death, and papillomacular bundle damage. If this process lasts longer then most likely will occur more bonds causing the increased risk of TON. However, this does not occur easily. Some factors are also influential and therefore a person might develop TON while the others do not. These factors include the use of other toxic substances such as isoniazid, chloroquine, chloramphenicol, amiodarone, cyclosporine, the use of these drugs are not found in the study subjects. Isoniazid, in particular, is reported to have toxic effects as on the eyes, but the incidence is very small. When starting this MDR-TB treatment program patients are not given isoniazid therapy because MDR-TB patients are resistant to isoniazid. However, most of the patients had received isoniazid therapy previously during the DOTS program. This could have an effect but is not analyzed. In addition, systemic disorders such as kidney and liver disorders that are likely to cause drug accumulation have also been excluded. Other factors that are also influential and not examined in this study are malnutrition conditions, and zinc deficiency.
Conclusion

There is a correlation between duration of ethambutol therapy with toxic optic neuropathy in patients with MDR-TB.

Conflict of Interest: There is no conflict of interest.

Source of Funding: This study is self-funded.

Ethical Clearance: This study was approved by Ethical Commission of Health Research Faculty of Medicine University of Airlangga.

References


Macrophage Activity and Histopathological Differences of Lung Tissue on Sequential Co-infections of Heligmosomoides Polygyrus Nematode on Mycobacterium Tuberculosis Infection

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Abstract

Background: Tuberculosis is a chronic infection caused by Mycobacterium tuberculosis, a facultative intracellular parasite, that can be eliminated by cellular immunity played by macrophages. It has become a debate whether the co-infection of nematodes will affect the immune response of macrophages towards mycobacterium infection.

Objective: To reveal macrophage activity and histopathological difference of lung tissue in sequential co-infection of Heligmosomoides Polygyrus towards Mycobacterium tuberculosis infection.

Method: This study used 49 mice divided into 7 treatment groups with Mycobacterium tuberculous infection by inhalation and Heligmosomoides polygyrus orally within 8 and 16 weeks, and observed by immunohistochemical staining.

Result: Infection for 8 weeks showed polarization of macrophages towards M1 macrophage, whereas in 16 weeks, the macrophage polarization more towards M2 macrophages, supported by histopathological changes of lung tissue: peribronchiolitis, perivaskulitis, alveolitis, and granuloma formation with counts of acid-resistant germs +3. There was a difference of expression of arginase1 to each group (p <0.001) and there was a difference of T CD4+ Th1 lymphocyte (p <0.001).

Conclusion: There is a difference in macrophage activity in lung tissue; however, it does not cause different levels of histopathological changes in lung tissue and does not affect the immune response to Mycobacterium tuberculosis infection.

Keywords: Heligmosomoides polygyrus, Mycobacterium tuberculosis, macrophage, Immunohistochemistry

Introduction

Tuberculosis (TB) is a chronic infection caused by Mycobacterium tuberculosis. According to WHO report in early 2012, it is estimated that 8.7 million individuals in the world suffer from TB infection especially in developing and low income countries. Most areas of the country with high TB incidence and low BCG vaccination effectiveness are also areas of high prevalence of worm infections ²-⁴. Worm infections cause changes in the immune response that harm the body’s defenses against TB infection ⁵,⁶.

Each year, there are 8.7 million new TB cases, with a mortality rate of around 1.4 million per year ¹. Mycobacterium tuberculosis is a paracitic intracellular facultative bacillus ⁷. An appropriate immune response to
eliminate TB is cellular immunity played by macrophages; CD4+ T-lymphocytes that secrete IFN-g; CD8+ T lymphocytes that eliminate infected macrophages with TB germs; as well as Tgd lymphocytes. This response requires a strong Th1 type cytokine. In contrast, worm infections stimulate the activation of eosinophil cells, mast cells, basophile cells, and IgE formation, which are Th2-type immune responses. The dominant Th2-type immune responses suppress the Th1 type immune response through suppression by IL-4.

Sequential research is certainly not ethical in human populations, because it can only be conducted with the standard model of nematode worm infection in mice that is Heligmosomoides polygyrus, and Mycobacterium tuberculosis sequentially. To describe the chronicity of a worm infection requires an interval of infection for at least 8 weeks. Chronic worm infection is known to trigger the onset of regulatory T cells (Treg) 11,12. Treg may affect the balance of Th1 and Th2 immune responses. Th1-Th2 balance will also affect macrophage function in overcoming mycobacteria infection 13,14. If it is proven that chronic infection of the worms stimulates the onset of Treg cells that are capable of altering the balance of Th1 - Th2 type immune responses and macrophage functional activity, then the debate about the effect of worm infection on histopathological changes in TB infection will be resolved. This study aimed to identify the effect of sequential co-infection of Heligmosomoides polygyrus nematodes on pulmonary histopathological changes in Mycobacterium tuberculosis infection 15.

**Method**

The research was conducted for 6 (six) months at Experimental Animal Cage of the Clinical Parasitology Division, Faculty of Medicine, Universitas Brawijaya and in Bacteriology Laboratory of Tuberculosis Infection Study Group of Tropical Diseases Institution, Universitas Airlangga, Surabaya, Indonesia. The research sample used 49 male (Mus musculus) mice of wild type aged 8-12 weeks with body weight of 30-35 grams. The sample was divided into 7 groups consisting of: a group infected with tuberculosis (TB) for 8 weeks (M.tb8), a group infected with TB (Mycobacterium tuberculosis) for 16 weeks (M.tb16), a group infected with a worm (Heligmosomoides polygyrus) for 8 weeks (H.pg8), a group infected with a worm (Heligmosomoides polygyrus) for 16 weeks (H.pg16), the group of mice treated with helminth co-infection (Heligmosomoides polygyrus) followed by TB infection (Mycobacterium tuberculosis) (H.pg + M.tb), a group of mice treated with TB co-infection (Mycobacterium tuberculosis) followed by a helminth infections (Heligmosomoides polygyrus) (M.tb + H.pg), as well as control group without infection treatment 16. Prior to conducting the research, the researchers conducted ethical test (151-KE) at the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.

**Result**

The activity of alternatively activated macrophage (AAMΦ) also known as M2 macrophage was characterized by the expression of Arginase1 protein. It was explained that macrophages with brown cytoplasm and Arginase1 protein were blue (arrows) and seen with a 400x light magnification microscope. Group 1: H. polygyrus infection for 8 weeks; group 2: H. polygyrus infection for 16 weeks; group 3: H. polygyrus infection for 16 weeks + M. tuberculosis for 8 weeks; group 4: infection of M. tuberculosis for 16 weeks + H. polygyrus for 8 weeks; group 5: 16 weeks of tuberculosis infection; group 6: M. tuberculosis infection for 8 weeks (figure 2). The level of Arginase 1 expression by macrophages in lung tissue showed in Table 1.

Histopathologic features and iNOS expression rates indicated that M. tuberculosis infection for 8 weeks resulted in infiltration of large amounts of macrophages into the infected lung tissue of mice and most of the macrophages infiltrating the tissue express iNOS (macrophage M1) activated in the atmosphere of Th1 cytokine. In infection with M. tuberculosis for 16 weeks, the number of macrophages that infiltrated the lung tissue was relatively decreased, and the level of iNOS expression in the macrophage group (4) whereas, histopathological features and levels of Arginase 1 expression indicated that M2 macrophages were also present in lung tissue, either in M. tuberculosis infection for 8 weeks or for 16 weeks. However, the level of Arginase1 expression by macrophages in mice for 8 weeks of M. tuberculosis infections (Fig. 3.-D) was lower than in the 16 weeks group of M. tuberculosis infections (Figure 3.-E).

To ascertain whether the duration of M. tuberculosis infection affected the level of expression of iNOS and Arginase1 by macrophages in lung tissue, MANOVA test was performed. From the statistical calculation, Box’s Test of Equality of Covariance Matrices obtained p value = 0.148 (p >0.05) which means homogeneous
data. From the Multivariate Test table on Hotelling’s Trace, \( p = 0.00 \) (\( p < 0.05 \)) showed that the duration of M. tuberculosis infection significantly affected the level of expression of iNOS and Arginase1 by macrophages in lung tissue.

**Histopathological Changes of Lung Tissue**

The calculated percentage of T CD4\(^+\) Th1 lymphocytes in peripheral blood showed a significant difference (\( p = 0.000 \)). The percentage of CD4\(^+\) T1 T lymphocytes in the highest lung tissue was found in M. tuberculosis infection for 8 weeks (\( 4.508 \pm 0.947 \)) and then decreased in M. tuberculosis infection for 16 weeks (\( 2.058 \pm 0.845 \)). The group treated with the last co-infection in the form of M. tuberculosis infection had a significantly higher percentage of CD4\(^+\) Th1 T lymphocytes than the opposite co-infection. In addition to assessing histopathological changes in pulmonary tissue, it was also calculated the acid-resistant M. tuberculosis bacteria in the lung tissue sieves preparation stained with Ziehl Neelsen staining (Table 1).

There was a correspondence between germination scores and histopathological lung rate change scores, wherein *H. polygyrus* co-infection did not affect acid-resistant bacteria count nor the rate of histopathological changes of lung tissue. The correlations between polarization of macrophage activity in lung tissue (ie, iNOS or Arginase1 expression) with histopathologic lung tissue change rate (ie Dormans scale score and acid-resistant bacteria count) were evaluated by Spearman test (Table 2).

**Table 1. Levels of arginase expression1 by macrophages and the number of M. tuberculosis in lung tissue**

<table>
<thead>
<tr>
<th>Group</th>
<th>Arginase1 Expression</th>
<th>CDC/ATS</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Min-Max</td>
<td></td>
</tr>
<tr>
<td>H.pg 8</td>
<td>19.20±0.45c</td>
<td>19.00-20.00</td>
<td>-</td>
</tr>
<tr>
<td>H.pg 16</td>
<td>23.40±1.14d</td>
<td>22.00-25.00</td>
<td>-</td>
</tr>
<tr>
<td>H.pg + M.tb</td>
<td>10.00±1.41b</td>
<td>9.00-12.00</td>
<td>+3</td>
</tr>
<tr>
<td>M.tb + H.pg</td>
<td>25.00±2.00d</td>
<td>23.00-28.00</td>
<td>+3</td>
</tr>
<tr>
<td>M.tb 16</td>
<td>25.20±1.64d</td>
<td>24.00-27.00</td>
<td>+3</td>
</tr>
<tr>
<td>M.tb 8</td>
<td>8.80±0.45b</td>
<td>8.00-9.00</td>
<td>+3</td>
</tr>
<tr>
<td>Control</td>
<td>3.80±0.45b</td>
<td>3.00-4.00</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: H.pg = *H. Polygyrus* infection; M.tb = M. tuberculosis infection; 8 and 16: infection for 8 and 16 weeks; The letters a, b, c, d: indicate that groups with the same letter marks have insignificant differences, whereas groups with different letter marks have significant differences.

**Table 2. The correlation between macrophage activity and histopathologic changes**

<table>
<thead>
<tr>
<th>Correlation between variables</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>iNOS and Dormans scores</td>
<td>0.523</td>
<td>0.001 *</td>
</tr>
<tr>
<td>Arginase1 and Dormans score</td>
<td>0.312</td>
<td>0.068</td>
</tr>
<tr>
<td>iNOS and acid-resistant bacteria count</td>
<td>0.723</td>
<td>0.000 *</td>
</tr>
<tr>
<td>Arginase1 and acid-resistant bacteria count</td>
<td>0.228</td>
<td>0.188</td>
</tr>
<tr>
<td>Dormans score and acid-resistant bacteria count</td>
<td>0.875</td>
<td>0.000 *</td>
</tr>
<tr>
<td>iNOS and Arginase1</td>
<td>0.058</td>
<td>0.739</td>
</tr>
</tbody>
</table>

*\( p < 0.05 \)
Discussion

In this study, we found an increase in the percentage of Th1 lymphocytes in lung tissue and in peripheral blood that correlated strongly with levels of IFN-γ cytokines in peripheral blood serum. The increase occurred in M. tuberculosis infection for 8 weeks which then ‘subside’ at the time of infection lasted up to 16 weeks. Our results are consistent with the results of several other researchers who found that elevated IFN-γ levels were primarily obtained in the early stages of the infection especially after the second week post infection. The mobilized lymphocytes accumulate at the site of infection, proliferate and secrete cytokines, especially IFN-γ. Protective immune responses to M. tuberculosis are more necessary for the role of Th1 type cytokines. Th1 type cytokines, including IFN-γ, are required not only to activate macrophages but also to assist the activity of CD8+ T lymphocytes.

Worm infection induces the emergence of T regulatory lymphocytes (CD4+ CD25+ Foxp3+) in both the intestinal tract, peripheral blood and lung tissue. T regulatory activity can be assessed from elevated levels of IL-10 and TGF-β cytokines in peripheral blood serum, as well as T regulatory lymphocyte percentage in intestinal tissue, lung tissue and peripheral blood. Similarly, the findings of the Th1 and Th2 lymphocyte immune responses, the T regulatory lymphocyte response were only found in the worms infection group for up to 8 weeks. When the infection has lasted up to 16 weeks, the activity and the role of T regulatory lymphocytes also decreased.

Activation of T regulatory lymphocytes apparently also occurs in M. tuberculosis infections, especially in infections lasting up to 8 weeks. Thus, it can be concluded that the time interval between the infection of the nematode worms and tuberculosis, as well as the observation will greatly affect the outcome of the co-infection. The success of the macrophage immune response is influenced by the balance of iNOS and Arginase expression which describes the direction of polarization of macrophage activity. Worm infections induce Th2-type immune responses, such as IL-4 and IL-13, leading to macrophage polarization into M2 macrophages expressing Arginase1. The purpose of M2 macrophage activation is to evoke an anti-parasitic response and repair tissue damage. In the group who received the last co-infection treatment of M. tuberculosis, the Arginase1 expression decreased whereas iNOS expression increased sharply.

This can be explained through several arguments that sequential infection of M. tuberculosis and H. polygyrus at intervals of 8 weeks does not affect the balance of T lymphocyte activity in lung tissue, sequential infection of M. tuberculosis and H. polygyrus at intervals of 8 weeks does not affect the ability of macrophages to generate an appropriate immune response for M. tuberculosis infection in lung tissue, and histopathological changes occurring in lung tissue due to M. tuberculosis infection are slowly changing and evolving changes that cannot be detected in the observation process for 8 or 16 weeks.

Immune responses to M. tuberculosis often fail to eliminate germs because germs are able to use several ways to circumvent the host’s immune response, among others by inhibiting the maturation and acidification of the phagosome, inhibiting the fusion of phagosome and lysosome, and escaping from the phagosome. It triggers macrophages to work together with CD4+ and CD8+ T lymphocytes to form granulomas that aim to isolate M. tuberculosis. However, M. tuberculosis can still survive in macrophages and beyond macrophages in granulomas. Thus, granulomas are retained for long periods of time through delayed-type hypersensitivity (DTH) responses requiring Th1 lymphocyte competence. If there is a significant decrease in immune response as well as in patients with HIV-AIDS macrophage, it can decay that allows dissemination of M. tuberculosis.

Conclusion

There is a difference in macrophage activity in lung tissue; however, it does not cause different levels of histopathological changes in lung tissue and does not affect the immune response to Mycobacterium tuberculosis infection.

Conflict of Interest: There is no conflict of interest.

Source of Funding: This study is self-funded.

Ethical Clearance: This study was approved by Ethical Commission of Health Research (151-KE) at the Faculty of Veterinary Medicine, University of Airlangga, Surabaya, Indonesia.

References

2. Fine PEM. Variation in protection by BCG:


Nurses’ Knowledge Regarding Pneumonia in Children Under Five Years of Age at Pediatric Wards in Kirkuk Teaching Hospitals

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Abstract

The Objective: to identify the nurses’ knowledge regarding pneumonia in children under five years of age at pediatric wards in Kirkuk teaching hospitals.

The Methodology: A descriptive study was carried out at pediatric ward of Teaching Hospitals in Kirkuk City to Assessment of Nurses’ knowledge regarding pneumonia in children under five years of age at pediatric wards from 20 of February till 3ed of June 2019. A non-probability (purposive) sample was chosen for the current study. The sample consisted of (40) nurses working in the pediatric medical ward of selected hospital.

The Result: The socio-demographic characteristic of the study presents that 62.5% of them females, 60.0 % at age (20 – 25) years old, 40.0 % graduated from college of nursing (Bachelor of Nursing) , 80.0% of them have (1-5) years of general experience. The results of the study sample presents (50.0 %) the majority of the nurses have poor knowledge in total knowledge score of pneumonia in Children under age five years. Also the mean of total knowledge score nurses in concern to protein energy malnutrition was (1.55) with standard deviation (0.714) . Shows that there were significant differences between the age and the service in the field of nursing with nurse’s knowledge but there were no significant differences between other parts of socio- demographic characteristics with nurse’s knowledge about pneumonia in children under age five years at p≥0.05 value.

The Recommendations: A continuous extensive special programs, training course and workshops regarding pneumonia in children under age five years should be design and implement in hospital’s departments to enhance the nurse’s knowledge in regard to this major issue.

Keywords: Assessment, Nurse’s knowledge, Pneumonia in children.

Introduction

Nursing education and training is progressing remarkably and requires a high level of attention in assessment methods, such as problem solving and critical thinking.¹ Pneumonia is a major cause of childhood morbidity and mortality especially in developing countries, particularly in developing countries; many studies have been conducted using population-based or hospital-based surveillance to estimate the disease burden of pneumonia². However, globally there is substantial variability in access to hospital treatment of childhood respiratory diseases including pneumonia at national and regional levels and within the healthcare systems³. It is estimated that about 0.9 million deaths happened due to pneumonia out of the total 6.3 million under-5 deaths in this population.
in 2013.(4,5). The pneumonia in high-income countries is different than in low-income countries, including more viral and atypical organisms causes(6,7). Pneumonia is a common serious infection that afflicts children less than five years throughout the world and is considered the most important global cause of death among them. It is an acute illness caused by infection. The lungs become inflamed, congested leading to cough and breathlessness. Nurse plays an important role in providing nursing care and health education for children and their families especially if they are skilled and well cultured.

**Objectives of the Study**

1. To assess the nurses’ knowledge level regarded pneumonia in children under five years of age at pediatric wards in Kirkuk teaching hospitals.

2. To find out the relationships between the socio-demographic variables and the nurses’ knowledge level regarded pneumonia in children under five years of age at pediatric wards in Kirkuk teaching hospitals.

**Material and Method**

**Design of the study:** A descriptive study was carried out at pediatric ward of Kirkuk Teaching Hospitals to assess of nurses’ knowledge regarded pneumonia in children under five years of age from 20ed of February till 3ed of June 2019.

**Sample of the study:** A non-probability (purposive) sample was chosen for the current study. The sample consisted of (40) nurses working in the pediatric medical ward of selected hospital. This is due to shortage of nursing staff and high number of children patients in the ward.

**Study tool:** The questionnaire was constructed and provided for nurses to assess the knowledge which consists of four parts. The first part concerns the social and demographic information, while the second part was related to the nurses’ knowledge in concern to pneumonia in children less than five years of age in general. The third part of the questionnaire dealt with nurse’s knowledge in about risk factors and medical management of pneumonia in children under five years of age, while the part four put emphasis on the nurse’s knowledge about nursing care management of pneumonia in children under five years of age.

**Validity of the study:** The validity of the questionnaire tool was established through a panel of experts whom specified the content clarity, relevancy, and adequacy.

**Reliability of the study:** To evaluate statistically the reliability of instruments, a pilot study was carried out during the period from 17 till 25 of March / 2019. Randomly (5) nurses were selected from Children’s Teaching Hospital (this sample was excluded from the original study sample). The Pearson’s coefficient of correlation result are ($r= 0.671$) and are significant at $p \leq 0.000$ level was used to estimate the scale (test – retest) by using SPSS version 20.

**Data collection:**

The data were collected from the selected hospitals in Kirkuk City. The study connected at the pediatric medical ward that available in the Kirkuk General Teaching Hospital and Children’s Teaching Hospital, at the period from 1st till 22 of April / 2019.

**Result**

The results shows that the demographic characteristics of the study sample, 60.0 % at age (20 – 25) years old, 62.5% of them females, 40.0 % graduated from college of nursing (Bachelor of Nursing), 80.0% of them have (1-5) years of general experience, 97.5% of them have (1-5) years of experience inside the medical pediatric ward , and lastly 57.5 not have any participation in training courses at the pneumonia in children under five years of age. Table (1) presents the results of Nurses’ Knowledge that 50.0% of them have not acceptable knowledge about pneumonia in children under five years of age in general, 40.0 % of them have Failure knowledge in relation risk factors and medical management of pneumonia in children under five years of age, 45.0 % of them have acceptable knowledge about to nursing care management of pneumonia in children under five years of age, and at lastly 50.0% of them have not acceptable knowledge in total knowledge score for all domains. Table (2): Shows that there were no significant differences between the most of social and demographic characteristics result with nurse’s knowledge regarding pneumonia in children under five years of age except the age with nurses knowledge level about pneumonia in children under five years of age in general, and nurses knowledge level about nursing care management of pneumonia in children under five years of age. also the service in the field of nursing with nurses knowledge level about risk factors and medical management of pneumonia in children under five years of age...
of age and nurses knowledge level about nursing care management of pneumonia in children under five years of age that there were significant differences at \( p \geq 0.05 \) value.

**Table (1): Nurse’s Responses Results in Concerning The Pneumonia in Children under Five Years of Age Knowledge.**

<table>
<thead>
<tr>
<th>No</th>
<th>Nurses Knowledge Level</th>
<th>Freq.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Nurses Knowledge Level about pneumonia in children under five years of age</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Failure</td>
<td>4</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>Not acceptable</td>
<td>20</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td>Acceptable</td>
<td>13</td>
<td>32.5</td>
</tr>
<tr>
<td></td>
<td>Good</td>
<td>3</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>Excellent</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>2.</td>
<td>Nurses Knowledge Level about risk factors and medical management of pneumonia in children under five years of age</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Failure</td>
<td>16</td>
<td>40.0</td>
</tr>
<tr>
<td></td>
<td>Not acceptable</td>
<td>15</td>
<td>37.5</td>
</tr>
<tr>
<td></td>
<td>Acceptable</td>
<td>4</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>Good</td>
<td>4</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>Excellent</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>3.</td>
<td>Nurses Knowledge Level about nursing care management of pneumonia in children under five years of age</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Failure</td>
<td>9</td>
<td>22.5</td>
</tr>
<tr>
<td></td>
<td>Not acceptable</td>
<td>9</td>
<td>22.5</td>
</tr>
<tr>
<td></td>
<td>Acceptable</td>
<td>18</td>
<td>45.0</td>
</tr>
<tr>
<td></td>
<td>Good</td>
<td>4</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>Excellent</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>4.</td>
<td>Total Nurses’ Knowledge Score for all domains regarding pneumonia in children under five years of age</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Failure</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>Not acceptable</td>
<td>20</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td>Acceptable</td>
<td>15</td>
<td>37.5</td>
</tr>
<tr>
<td></td>
<td>Good</td>
<td>4</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>Excellent</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>40</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Table (2): Statistical Differences of Social and Demographic Characteristics Result of Nurses Knowledge Level about Pneumonia in Children under Five Years of Age.

<table>
<thead>
<tr>
<th>Domains</th>
<th>Nurses Knowledge Level about pneumonia in children under five years of age in general</th>
<th>Nurses Knowledge Level about risk factors and medical management of pneumonia in children under five years of age</th>
<th>Nurses Knowledge Level about nursing care management of pneumonia in children under five years of age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T</td>
<td>Sig.</td>
<td>T</td>
</tr>
<tr>
<td>Age</td>
<td>1.309</td>
<td>0.079</td>
<td>0.805</td>
</tr>
<tr>
<td>Gender</td>
<td>0.588</td>
<td>0.560</td>
<td>0.725</td>
</tr>
<tr>
<td>Education Level</td>
<td>0.982</td>
<td>0.333</td>
<td>0.162</td>
</tr>
<tr>
<td>The service in the field of nursing</td>
<td>0.080</td>
<td>0.937</td>
<td>2.544</td>
</tr>
<tr>
<td>The duration of active duty in the unit esoteric</td>
<td>0.326</td>
<td>0.747</td>
<td>0.313</td>
</tr>
<tr>
<td>The participation in training courses</td>
<td>0.644</td>
<td>0.524</td>
<td>2.508</td>
</tr>
</tbody>
</table>

Discussion

The demographic characteristics of the study of total sample number (40)N, presents that 60.0 % at age (20 – 25) years old, 62.5% of them females, 40.0 % graduated from college of nursing (Bachelor of Nursing), 80.0% of them have (1-5) years of general experience, 97.5% of them have (1-5) years of experience inside the medical pediatric ward, and lastly 57.5 not have any participation in training courses at the pneumonia in children under five years of age. This result agree with Eiman M. ; Oshak S., (2016) They presented that the nurses’ ages ranged between 20 - 30 years. Their education levels were varied with bachelor level (76%) and the diploma (24%) and post graduate level (0%). So there was an increase in knowledge about pneumonia with an increase educational level. also revealed that nurse’s years of experience >2 year (54%) and 2-3 year (20%) and> 4 year (26%) was not related to the quality of care. Therefore, the common worker in this hospital was less experienced as they were practicing only for 2 years(8). Nurse’s Responses Results in concerning the pneumonia in children under five years of age Knowledge. The results of nurses’ knowledge level presents that 50.0% of them have not acceptable knowledge about pneumonia in children under five years of age in general, 40.0 % of them have Failure knowledge in relation risk factors and medical management of pneumonia in children under five years of age, 45.0 % of them have acceptable knowledge about to nursing care management of pneumonia in children under five years of age , and at last  50.0% of them have not acceptable knowledge in total knowledge score for all domains Table (1). These results agree with Mogahed, (2011) his result showed that: (25.0%) was poor, (57 .0%) fair, (17 .0%) had good knowledge of participants’ regarding prevention of pneumonia. Therefore, factors such as level of qualification, and experience years, does not significantly affect level of knowledge(9). Statistical differences of social and demographic characteristics result of nurses’ knowledge level about pneumonia in
children under five years of age. The result of study shows that there were no significant differences between the most of social and demographic characteristics result with nurse’s knowledge regarding pneumonia in children under five years of age. Except the age with nurses knowledge level about pneumonia in children under five years of age in general, and nurses knowledge level about nursing care management of pneumonia in children under five years of age. Also the service in the field of nursing with nurses knowledge level about risk factors and medical management of pneumonia in children under five years of age and nurses knowledge level about nursing care management of pneumonia in children under five years of age that there were significant differences at P ≥ 0.05 value Table (2). This result agree with Tahseen A. (2015) was a significant association between nurses’ knowledge and their age and years of employment. This means that nurses’ ages and years of employment have great impact on their knowledge(10). But This result disagree with AL-Sa’idi, B.: (2006) and Shuq, A. H (2008) they showed that was a significant association between nurses’ knowledge and demographic characteristics(11,12).

**Conclusion and Recommendations**

According to the results of the present study, the researcher concludes the nurses do not have appropriate and adequate knowledge about the pneumonia in children less than five years of age. There is significance relationship between the nurse’s knowledge and age and the service in the field of nursing toward the pneumonia in children under five years of age only. But there is no significance relationship between the nurse’s knowledge and other socio-demographic characteristics toward the pneumonia in children under five years of age. According to the results and conclusion of the present study, the researchers recommend the Kirkuk Health Directorate has to conduct statistics of certified prevalence about pneumonia in children under five years of age at all pediatric hospitals at Kirkuk City on the web site. Also special programs, training course and workshops about child pneumonia in general, risk factors, medical management, nursing care management can be designed and implemented in hospital’s departments to enhance the nurse’s knowledge in regard to this major issue. Increasing the number of nurses especially those who hold Bachelor degree in nursing to work at the pediatric medical wards for their ability and background.

**Ethical Consideration:** Prior to data collection, official permission was obtained from the Ministry of Health/ Department of Planning and Health Research committee and Written approval of participants was obtained prior to the start of data collection.

**Conflicts of Interest:** Nil

**Source of Funding:** Self.

**Acknowledgment:** This research was partially supported by College of Nursing at University of Mosul, Iraq. We are thankful to our colleagues who provided expertise that greatly assisted the research, although they may not agree with all of the interpretations provided in this paper.

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The Effectiveness Comparison of Desloratadine and Loratadine in Reducing Total Nasal Symptom Score and the Level of Interleukin 4 in the Nasal Secretions of Allergic Rhinitis Patients

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¹Department of Otolaryngology, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo Teaching Hospital, Surabaya, 60285, Indonesia

Abstract

**Background:** Allergic rhinitis (AR) is a common atopic disease; however, the available therapy has limitation in the usage and its success rate. Desloratadine has a role as an alternative allergic rhinitis therapy to eliminate the overall symptoms of AR and reduce IL-4 level in nasal secretions.

**Method:** This study was double-blind randomised clinical trial with pre-post test design. The data was analyzed by using independent sample t-test. There were 24 AR patients divided into 2 therapy groups which were study group (desloratadine) and control group (loratadine). The evaluation based on SGHT and IL-4 in nasal secretions was conducted pre and post 15 days of therapy.

**Results:** The present study that was conducted on July-October 2016 showed significant difference in the average percentage of reduced TNSS in desloratadine group compared to loratadine group (p=0.000). The comparison of reduced level of IL-4 in nasal secretions between desloratadine and loratadine group was indicated insignificantly different.

**Conclusion:** Reduced TNSS in desloratadine group was higher than in loratadine group. Also, there was no difference in decreased level of IL-4 in nasal secretions.

**Keywords:** Desloratadine, loratadine, total nasal symptom score, interleukin 4, allergic rhinitis.

Introduction

Allergic rhinitis (AR) is a common inflammatory nasal mucosal disease mediated by Immunoglobulin E (Ig E) after the exposure of allergen in nasal mucosa. The main symptoms of AR include sneezing, stuffy nose, runny nose, and itchy nose. Several cases of AR are accompanied by the occurrence of symptoms in eyes, ears, and post nasal drip in throat. The assessment of AR symptom severity is determined by total nasal symptom score (TNSS). The clinical manifestations of AR involve proinflammatory cytokines and other cytokines; one of the essential cytokines in AR is Interleukin 4 (IL-4) which has a role to form Ig E and recruit eosinophils.

Loratadine is an H1-antihistamine that has not been able to eliminate the overall symptoms of AR; therefore, the use of loratadine is often combined with other drugs. Desloratadine is an active metabolite from loratadine known to have more potent affinity towards H1 receptor than loratadine itself. It also can reduce the level of IL-4 in AR. The high severity of AR encourages researchers to reveal other AR therapies beside loratadine. However, it has not been conducted any study that compares the effectiveness of desloratadine and loratadine in reducing TNSS and IL-4 level of nasal secretions in AR patients.
The latest research proved that desloratadine was an active metabolite from loratadine and as a single therapy to eliminate AR symptoms especially stuffy nose and reduce IL-4 level in nasal secretions. Desloratadine is the main active metabolite from loratadine that can decrease either nose symptoms or other symptoms such as chronic idiopathic urticaria. Another in vitro research also showed that the affinity of desloratadine towards H1 receptor was 50-194 times bigger than loratadine, cetirizine, and fexofenadine. Another study also stated that in 2 weeks, AR patients who consumed desloratadine 5mg/day has a significant impact in decreasing stuffy nose than placebo (p<0.05).

One of the cytokines from Th2 that plays an important role in AR pathophysiology is IL-4. The role of IL-4 is to produce and differentiate B cell to be plasma cell. Afterwards, it will produce Ig E that is tied to mast cell and basophile. In addition, another role of IL-4 is to competitively impede or produce cytokines from Th1. It also has a role in recruiting eosinophil which is connected to vascular cell adhesion molecule-1 (VCAM-1). The activation of eosinophil causes chemical mediator extrication such as mayor basic protein (MBP), eosinophil cationic protein (ECP) and eosinophil peroxidase (EPO). This chemical mediator causes clinical manifestation such as sneezing, runny nose, itchy, and stuffy nose.

A particular research revealed that the total of IL-2, IL-4, IL-13 cytokines and TNF-α in nasal secretions was significantly higher (p<0.05) rather than the total number in serum. IL-4 significantly increases in AR patients compared to non AR patients. Based on the elaboration above, the researcher is intended to conduct a study to compare the effectiveness of desloratadine and loratadine to reduce TNSS and IL-4 level in nasal secretions in AR patients.

**Method**

The research subjects were moderate-severe intermittent AR patients, mild intermittent AR patients and mild persistent AR patients based on ARIA 2008 who were treated in Outpatient Otolaryngology-Head and Neck Surgery Unit, Division of Immunology-Allergy, Dr. Soetomo General Hospital Surabaya and fulfilled inclusion and exclusion criteria. The inclusion criteria were pre therapy TNSS more than or equal to 5, aged 21-60 years old, have not consumed anti allergic drugs such as: AH (1 week), systemic corticosteroids (4 weeks), topical corticosteroids (2 weeks), topical decongestants (1 week), anticholinergic and chromoline, willing to follow the research and signed the consent forms. The exclusion criteria were acute respiratory tract infections, acute and chronic paranasal sinusitis, medical rhinitis, nasal abnormalities such as tumors, rice polyps, severe deviation septum, pregnancy or lactating and a history of loratadine allergy.

This study was conducted by employing double-blind randomized controlled trial with pre-post test design. The sample collection was conducted by using the method of consecutive sampling on July-October 2016. The samples were allocated randomly based on block permutation technique which was divided into study group and control group. The study group obtained desloratadine therapy 5 mg/day while the control group obtained 10 mg/day. The samples were excluded from the study if they resigned from the research, underwent severe side-effects or dismissed by the researchers for patients’ own good, not consume the drugs for 2 (two) days in sequence, consumed sympathomimetic drugs, AH, systemic and local steroidal anti inflammatory, ketoconazole, erythromycin and cimetidine during the research, and not come to the medical control in the last evaluation on the fifteenth day.

The measurement of IL-4 level in nasal secretions was conducted by using nasal lavage. Each nasal cavity was inserted 5 ml of isotonic saline solution (it was warmed up in 37°C) by using squirt 10 cc. The supernatant was collected and stored in a refrigerator in a temperature of -80°C in Biomedical and Tissue Bank Unit Dr. Soetomo General Hospital Surabaya. The measurement and reading of IL-4 level in nasal secretion in the initial and last evaluation were conducted simultaneously. The statistical analysis on level of significance (p) of 0.05 or 5% applied 2 sample independent t-test to find out the comparison of desloratadine and loratadine therapy towards TNSS and IL-4 level in nasal secretions.
Results

Table 1. The severity scale of TNSS test

<table>
<thead>
<tr>
<th>No</th>
<th>Symptom</th>
<th>Scale</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Runny nose</td>
<td>0</td>
<td>no symptom</td>
</tr>
<tr>
<td>2</td>
<td>Stuffy nose</td>
<td>1 (mild)</td>
<td>symptoms that do not interfere</td>
</tr>
<tr>
<td>3</td>
<td>Sneezing</td>
<td>2 (moderate)</td>
<td>clear symptoms and signs, disturbing symptoms but still tolerable</td>
</tr>
<tr>
<td>4</td>
<td>Itchy</td>
<td>3 (severe)</td>
<td>symptoms and signs disturbed daily activities/sleep</td>
</tr>
</tbody>
</table>

Table 2. The comparison of reduced TNSS between Desloratadine group and Loratadine group

<table>
<thead>
<tr>
<th>Reduced TNSS</th>
<th>Group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Desloratadine</td>
<td>Loratadine</td>
</tr>
<tr>
<td>N</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Average</td>
<td>7,67</td>
<td>2,25</td>
</tr>
<tr>
<td>SB</td>
<td>1,68</td>
<td>1,48</td>
</tr>
</tbody>
</table>

Table 3. The comparison of reduced IL-4 level in nasal secretions between Desloratadine and Loratadine group

<table>
<thead>
<tr>
<th>Reduced TNSS</th>
<th>Group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Desloratadine</td>
<td>Loratadine</td>
</tr>
<tr>
<td>N</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Average</td>
<td>7,67</td>
<td>2,25</td>
</tr>
<tr>
<td>SB</td>
<td>1,68</td>
<td>1,48</td>
</tr>
</tbody>
</table>

There were 26 AR patients appointed as research samples. During the study, it was found 2 drop out cases which were 1 sample from study group on the fifteenth day and 1 sample from control group because the patient suffered from sore throat and had to consume other drugs. Therefore, the samples that can be analyzed were 24 patients. They were divided into two groups: 12 patients in study group and other 12 patients in control group. The male patients were 8 subjects while female patients were 16 subjects. The youngest age of those 2 groups was 21 years old while the oldest one in control group was 52 years old and in study group was 58 years old. The number of moderate-severe intermittent AR was 18 patients; mild intermittent AR was 3 patients; and mild persistent AR was 3 patients.

The result of statistical test using independent sample t-test was $p=0.000$ that indicated a significant different in the comparison of reduced TNSS between desloratadine and loratadine group (table 2). The result of statistical test using independent sample t-test was $p=0.256$ that indicated insignificant different ($p>0.05$) in the comparison of reduced IL-4 in nasal secretions between desloratadine and loratadine group (table 3). During the study, it was reported that the most frequent side-effect in both groups was sleepy which occurred in
Discussion

The result showed that the average reduced TNSS in desloratadine group was 7.67 (SB 1.77) while in loratadine group was 2.42 (SB 1.62). The statistical test using independent sample t-test obtained the value of p=0.000. The value indicated a significant different in the comparison of reduced TNSS between desloratadine and loratadine group (p<0.05). It can be concluded that desloratadine was more effective compared to loratadine in reducing TNSS in AR patients. Desloratadine is the main active metabolite from loratadine. Seen from pharmacology, desloratadine and loratadine have similar pharmacokinetic profile which work in a quick phase and slow phase, inhibit the histamine bond with its receptor, inhibit infiltration and eosinophil activation to eliminate several mediators such as MBP, ECP, EPO, and EDN, inhibit mediator release from mast cell and basophile through direct obstacle in calcium ion canal to prevent further damage of the nasal mucosal epithelium. Based on the data of preclinical study, desloratadine was 60 times more selective towards H1 receptor than H2 receptor. Also, it had no affinity towards receptors of dopamine, monoamine oxidase, acetyl-cholinesterase, δ-amino butyric acid, and bradykinin. In addition, loratadine is a selective tricyclic antihistamine towards peripheral H1 receptor and not indicate the activity towards H2 receptor

Based on preclinical study, the desloratadine affinity towards H1 receptor is 50-194 times higher than loratadine, cetirizine and feksonadine. QingJia conducted a study on January until October 2014 in AR patients who underwent desloratadine and loratadine therapy. It revealed that desloratadine had the therapy effects such as runny nose, stuffy nose, itchy nose, sneezing and better edema inferior than loratadine. The previous study had similarity with this study which is reduced TNSS in desloratadine is better than loratadine group.

The result of this study is not in accordance with the previous study. The double blind research in PAR patients who had allergic towards dermatophagoides farinea, cat’s and dog’s fur and obtained desloratadine 5 mg and levoceterizine 5 mg showed reduced IL-4 after 4 weeks therapy towards both drugs. Another study showed that the effect of desloratadine towards reduced IL-4 level in SAR patients after 2 weeks therapy with desloratadine 5 mg/day. A study in PAR patients also revealed that desloratadine reduced IL-4 in nasal rinses during four weeks of therapy.

The technique of nasal rinse collection in the previous study was conducted by provoking the previous allergen in order to determine the proper time to measure IL-4 level. The technique of nasal rinse collection in this study was not conducted. However, it was conducted in every patient with AR in order to recognize the difference of exposure time to allergens among the patients during sample collection. This condition can impact the measurement result of IL-4 level in various nasal secretions and we are not able to recognize the proper time to measure IL-4 level in nasal secretions.

The previous study indicated that IL-13 has a role in slow phase allergy in which an increase was obtained 24 hours after allergen provocation test. IL-13 can affect chronic stuffy nose symptom in AR patients. Another study in AR patients obtained increased IL-4 and IL-13 level in nasal secretions after undergoing the provocation test of pollen and wheat powder allergen. The administration of anti IL-4 antibodies during the sensitization phase shows an inhibitory effect in the development of Th2. This suggests that IL-4 is important in fast type antigen responses. If IL-4 is given to animals that encounter sensitization, it is less affected in reducing the production of Th2 cytokines and eosinophil reflux. On the other hand, IL-13 has more roles than IL-4 after secondary antigen exposure. The therapy implementation in allergic patients who has been given IL-4, IL-5 and IL-13 does not increase disease improvement and the symptoms caused by other mediators in allergic diseases.

The hypothesis of this study was not proven that desloratadine had bigger role in reducing IL-4 level in nasal secretions than loratadine in AR patients. It was because IL-4 is not the only factor affecting the occurrence of AR symptoms. Several mediators and proinflammatory cytokines derived from mast cell degranulation play an important role in the emergence of clinical symptoms in AR. The previous research reported that there was no association between nose clinical symptoms with several signs of inflammation in perennial AR. Other researches revealed that there was no significant association between the severity of nose symptoms and nose inflammation.
Conclusion

The present study can be concluded that desloratadine is more effective than loratadine in reducing TNSS; however, the effectiveness is similar in reducing IL-4 level in nasal secretions in AR patients. It is required to conduct allergen provocation test in order to determine the proper time to collect the samples to measure IL-4 level in nasal secretions. IL-4 has similar function to IL-13; therefore, it is required to conduct a research with IL-13 variable simultaneously. The result of desloratadine therapy which was significant in reducing TNSS can be considered as an alternative of AR therapy other than loratadine.

Conflict of Interest: There is no conflict interest

Source of Funding : This study is self-funded

Ethical of Clearance: This study was approved by Ethical Commision of Health Research Faculty of Medicine University of Airlangga

Reference


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Prevalence of Dermatophytes Fungal Infection among Different Gender

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Abstract

The present study was designed to determine the prevalence of superficial fungal infections among the patients that were living in different circumstances in Diyala region, Iraq. From July 2018 to February 2019, there were (218) patients with ages (2-78) years were surveyed for dermatophyte fungal infections by history and clinical examination. The results revealed that (71.2%) of the patients were infected with skin lesions followed by scalp lesions (16.5%) and nail lesions (14.6%). The prevalence rates of dermatophyte fungal infections were linked to the types of tinea infections, including tinea corporis (17.4%), tinea pedis (13.7%), tinea capitis (16.5%), tinea unguium (14.6%), tinea cruris (17.4%), tinea faciale (3.6%). The proportion of fungal infection in rural area (63.6%) was more preponderant in comparison to urban areas (39.8%). Furthermore, the rate of percent infection by tinea capitis (66.6%), tinea corporis (60.5%), tinea cruris (57.8%), were higher in male whereas the percentage of tinea unguium (87.5%) and tinea faciale (62.5%) infections were highest in female. In addition, study demonstrated that indices of tinea infection has been significantly associated with age. This study suggested there has been an exigent need to ameliorate the edification of hygiene values of peoples in addition to increment the economic condition of the society.

Keywords: Dermatophytes, fungal infection, living conditions

Introduction

Fungi are typical eukaryotic cells, representing a distinct kingdom, estimate one quarter of a million species, only few are pathogens to humans, or other warm-blood animals. Fungi may be broadly divided into two basic forms, the moulds and the yeasts. The moulds are made up of long multinucleated filaments called hyphae, which can infect the skin, hairs, nails and the internal organs. The yeasts are made up of unicellular ovoid to globes cell, which usually reproduced by budding or more rarely by fission, which can infect the skin, nails, mucous membrane and the internal organs. Fungal infections of the skin may be divided into two types according to the level of skin involvement, the superficial and deep infections. The superficial infections, which are confined to the stratum corneum, hairs and the nails, which include: dermatophytosis, candidiasis and other non-dermatophyte infections. Clinical manifestations of dermatophyte infections vary depending on the site of infestation and the type of strain; therefore, accurate identification of the strain is crucial in order to facilitate rapid treatment and to prevent spread of the disease.

The prevalence of different types of superficial fungal infections of the skin is variable among different age group, gender, socio-economic levels and countries, in UK and other Western Europe countries, the prevalence is 5.1% and in USA is 8.1% and in general it is more prevalent in male’s children and 5.In Iraq two studies was done, one in Baghdad and the other in Diyala province, showed that the prevalence was 9.9% of all skin diseases. Regarding the different types of superficial fungal infections of the skin, tinea capitis and corporis are more prevalent in males children aged 3-14 years and more in African countries Dermatophytes species belonging to three genera (Trichophyton, Microsporum, and Epidermophyton). Trichophyton and Microsporum genera are the most numerous and diverse, there are over 40 species belonging to these two taxonomic groups.
**Epidermophyton** genus has only one representative – *Epidermophyton floccosum* species. Transmission of dermatophytes may occur by direct contact with infected humans or animals or indirectly by contact with contaminated fomites. Dermatophytes require keratin for growth and they can cause superficial infections of the skin, hair, and nails. Dermatophytes may spread by direct contact from other people (anthropophilic organisms), animals (zoophilic organisms), and soil (geophilic organisms) as well as indirectly from fomites. The disease is widely distributed all over the world with various degrees and more common in men than in women.

So the aim of the present study is to demonstrated the correlation of gender, age, place of residence (rural or urban), and the nature of work practiced by the patient on dermatophyte infection.

**Materials and Method**

A sample size of Two hundred eighteen were collected from patients who have attended the dermatology unit at Baquba’a teaching Hospital, Baquba’a City, Diyala Province between first July 2018 to end February 2019. The association of frequency of dermatophytoses infection to the individual were investigated in terms of following aspects: place of residence (rural or urban); health history of the individual; marital status and the nature of work practiced. Diagnosis was made according to the diagnostic criteria listed by in terms of patient’s history and clinical examination.

**Results and Discussion**

Among the 218 cases, results (table 1) showed that the dermatophyte fungal infections were comprised from the followings types: Tinea capitis (16.5%), and Tinea faciale (3.6%), Tinea cruris (17.43%), Tinea corporis (14.6%), Tinea ungium (14.6%), Tinea pedis (13.7%).

Evidences suggested that ringworm infections are quit prevalent and found to be one of the most frequent causes of dermatological complications.

This difference may be attributed to the fact that the secretion of androgen peaks during adolescence and therefore sebaceous secretion increases. for flaring up but there is no significant correlation regarding species identified and age group. The current study disagrees with which differed from other studies results .In addition to Candidasis is more prevalent in adult females, pityriasis (tinea) versicolor is more prevalent in adult males.

**Table (1) : no. of tinea types among male , female and age groups**

<table>
<thead>
<tr>
<th>Age group</th>
<th>Tinea capitis</th>
<th>Tinea faciale</th>
<th>Tinea cruris</th>
<th>Tinea corporis</th>
<th>Tinea ungium</th>
<th>Tinea pedis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>Females</td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td>0 – 10</td>
<td>18</td>
<td>9</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>11 – 20</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>21 – 30</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>10</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>31 – 40</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>41 – 50</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>51 – 60</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>61 – 70</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>71 – 80</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>36/218</td>
<td>8/218</td>
<td>38/218</td>
<td>32/218</td>
<td>32/218</td>
<td>30/218</td>
</tr>
</tbody>
</table>
Distribution of dermatophyte fungal infections based on residence area

The result has shown that the highest ascendant of dermatophyte fungal infections within rural area (57.38%) compared to urban area (42.6%).

The results revealed that the highest ascendant of dermatophyte fungal infections within urban areas were Tinea cruris (68.4%), Tinea corporis (63.1%), Tinea unguium (62.5%), and Tinea pedis (63.3%), whereas in rural areas the percentage of these fungal infections were found lowest, all other types of tinea infections were substantially higher in rural areas as compared to urban areas.

Evidences additionally suggested that residential area (rural and urban) is known to markedly affect the rate of infection as the most of rural areas are inhabited by the families with animal husbandry such as canines and cattle which are the source of Tinea infections. These factors are further known to spread fungal disease in the region where health accommodations are circumscribed in comparison to urban areas and 15 and 16.

Some researchers additionally attributed the concept of self-pollination (autoinoculation) of ringworm types which have been known to be associated with climatic conditions of rural or urban area. Tinea capitis and Tinea faciale were significantly more common in rural area. In particular, Tinea capitis was more mundane in rural area as compared to urban area. One reasons for Tinea capitis infections in rural areas is associated with animal husbandry profession of the peoples. Other one due to over crowdedness in joint families which impart close contact with children and philic infectious agents 16.

Our results showed Infections of Tinea cruris were higher in urban places than in rural areas and this to be consistent with earlier findings as described by Figueroa et al., that skin diseases are the second most common cause for medical consultation for children in rural communities. It has additionally been shown that in regions with a poorer socioeconomic environment, the morbidity rates, especially with infectious diseases are found to be higher. The most significant difference is in tinea capitis (52.7%).

The education level of the patients in this study results showed that the percentage of infection by Tinea capitis (77.7%), Tinea corporis (52.6%), Tinea pedis (50%), and Tinea faciale (75%) were found to be highest in primary and secondary education, while the Tinea cruris (63.1%) had the highest rate of infection in university education (fig.1): This result comes in accordance with 19. There is an increased activity of sebaceous glands under the hormonal influence in adolescents and young adults and this explains the increased frequency of tinea infection among the student population 16.

On the other hand, 20 stated that military personnel, athletes, and those doing hard works that usually associated with hyper sweating were more vulnerable to pityriasis versicolor infection. Furthermore, sebaceous glands secretion is increased in males 15 years and older compared to females, and that may also promote, pityriasis versicolor infection in males.

![Figure (1) Distribution of patients with dermatophytes according to their education.](image-url)
The results in table (2) have revealed that the highest ascendant of dermatophyte fungal infections in child and students were Tinea capitis (100%), Tinea cruris (47.3%), while the Tinea corporis (47.3%) had the highest rate of infection in self employers and workers, and Tinea unguium (62.5%), Tinea pedis (70%) is to be highest in housewives because of using detergents. In addition, students are also more involved in sports activities which predisposes them to increased sweating and hence, pityriasis versicolor 21.

### Table (2) : tinea types distributed among different factors(study, self employed, housewife & salaried)

<table>
<thead>
<tr>
<th>Type of tinea</th>
<th>Child and student</th>
<th>Self employed And worker</th>
<th>House wife</th>
<th>Salaried</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tinea capitis</td>
<td>36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tinea faciale</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Tinea corporis</td>
<td>4</td>
<td>18</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>Tinea cruris</td>
<td>18</td>
<td>8</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Tinea unguium</td>
<td>1</td>
<td>4</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>Tinea pedis</td>
<td>0</td>
<td>5</td>
<td>21</td>
<td>4</td>
</tr>
</tbody>
</table>

The nature of the work could also affect the proportion of infection as our result revealed that 39.9% of infected patients were students whereas 33% patients were house wives and rest of 28% patients belongs to others diverse range of professions. The results also suggested that the marital status positively correlated with the proportion of dermatophyte infection.

Akcaglar reached to the same conclusion that the increasing occurrence of dermatophyte fungal infections could be attributed to living circumstances with increase the number of the people that living in close contiguity in addition to the patient’s family history, lifestyle, immune status. Prevalence of different types of fungal infections varied according to geographical location, environmental conditions, and cultural factors, age, gender, socioeconomic status and predisposition to diabetes amongst others 22 and 23.

Host susceptibility may be enhanced by moisture, warmth, concrete skin chemistry, composition of sebum and perspiration, heftily ponderous exposure and genetic predisposition. The incidence of fungal infection is higher in sultry humid climates and in crowded living conditions 22. The differences in the incidence of superficial infections between the age groups and genders may be reflected the differing rates of sebum production and fluctuations of immunity with aging 21.

The results have demonstrated that the highest ascendant of dermatophyte fungal infections in oily skin 107(60.79%), while 19 (10.79%) were dry, and 50(28%) were normal fig.2):

![Figure (2) Type of skin](image)

Unfortunately, why some people suffer from excessive sebum production while others endure dry skin remains difficult to explain. Numerous factors have been proposed to play a role in the pathogenesis of oily skin. Thus, pinpointing one successful treatment is challenging. Here, we review the physiology of sebaceous glands as well as current and up-and-coming treatment options that can be offered to patients.
Concerned about oily skin.\textsuperscript{23}

Despite the pessimistic view of sebaceous glands as a result of their role in oily skin, they do play a vital role in the skin’s well being. Sebaceous glands display endocrine function (particularly androgen synthesis), compose the fetal vernix caseosa, and play a key role in the epidermal barrier and innate immunity. Here, we review current and up-and-coming treatment options that may be utilized to help patients with oily skin.\textsuperscript{24,25} High level of sebum production in such age group which offer suitable condition to lipophilic Malassezia for flaring up but there is no significant correlation regarding species identified and age group.\textsuperscript{10}

Predisposing factors for the development of \textit{Malassezia} infection include excessive production of sebum and/or decreased quality of sebum (seborrhea), accumulation of moisture, damage of epidermis, concurrent dermatoses, atopy, and bacterial skin infections\textsuperscript{26}.

\textbf{Conclusion}

The present study demonstrated that the dermatophytosis was more common in educated people and in low socio-economic regions that is because crowded and bad hygiene characteristics of such community. The types of tinea are varies between tinea corporis and tinea cruris especially with the adult age group. Also, tinea capitis record high rate which found mainly in children. Further studies are urgently required to fully understand the pathomechanisms of the doggedness of dermatophyte fungal infection in certain patient population by using more sensitive and precise methods for example polymears chain reaction with specific primers [conventional polymerase chain reaction and real time polymerase chain reaction], restriction fragment length polymorphism ,in situ hybridization of fluorinated neucleotides, nested polymerase chain reaction .

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\textbf{Source of funding}- Self

\textbf{Conflict of Interest - None}

\textbf{References}


Risk Factors of the Elderly Falling in Public Hospitals: A Systematic Review Study

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Abstract

Background: Falls are the most common and problematic issue of old age. There is the possibility of falls among the elderly in the hospitals. The present study was conducted to determine the risk factors for falls in the elderly in hospitals through systematic review.

Method: This is a systematic review based on the PRISMA protocol. The IranMedex, SID, Magiran, IranDoc, Medlib, Pubmed, Scopus, Web of Science, Science Direct, Medline and Google Scholar databases were searched for keywords in the time frame of 1994-2017.

Results: The mean age was 64-81 years old and the number of elderly people varied from 88 to 28524 people. Of these studies, six studies were performed in the acute care unit, three studies in the elderly care unit, three studies in the surgical and clinical department, one study in the rehabilitation department, one study in the psychiatric department and other studies were performed in several parts of the hospital. The results of this study referred to individual and external factors as risk factors for the falls in the elderly in the hospital. The use of medications, physical problems, and psychological problems were the most common risk factors for falls in the elderly.

Conclusion: In acute care, chronic care, psychiatric care, and elderly care units, there are many risk factors for the falls in the elderly due to their physical and mental status. Identifying and resolving these risk factors may provide safety for the elderly.

Keywords: Risk Factors; Accidental Falls; Aged; Hospitals; Systematic Review.

Introduction

Elderly is a stage of life influencing a person’s biological, mental, and physical status (¹). Enhancing the quality of medical care is one of the main concerns on the part of healthcare professionals. A major goal of a healthcare organization can be the prevention of any harm to patients and avoiding endangering patients’ safety following providing healthcare for them (²). Diseases that threaten this group include diabetes and hemodialysis³, ⁴.

There are many types of trauma, including vascular trauma, accidents, and falls⁵-⁷. Falling often put people over 65 at risk influencing their economic, social, and psychological status such as losing the sense of independence and self-assurance. These can, in turn, ensue negative effects which may culminate in fracture or even their death⁸. Moreover, these factors can make the elderly vulnerable to various complications including fracture, destruction of soft tissues, bruises, and subdural hematoma⁹. Falling can also have different physical consequences (such as hip fracture, disablement, and inability to move) and mental consequences (including losing one’s self-confidence, self-esteem, and lowering of life expectancy)¹⁰. It ought to be noted that falling not only influences the patients’ lives, but also it may lead to the prolonging of hospitalization period as well as healthcare expenses in medical care system¹¹.

The major risk factors affecting falling are categorized into four groups: 1) biological factors such as age, gender, diseases, cognitive disabilities; 2) socio-economic factors including literacy, income, housing, public health, and social seclusion; 3) behavioral factors
such as fear of falling, life style, concomitant taking of medications, doing no exercise, and unsuitable clothing; 4) environmental factors like building planning, stairs, slippery halls and floors, carpets, floorings, fences, and bathroom (12, 13). One of the tasks of nurses is to improve patients’ health (14). The current study seeks to identify risk factors for the elderly falling in hospitals through a systematic review method.

**Material and Method**

The present study is a systematic review which attempts to examine risk factors underlying the elderly falls in hospitals around the world. In this study PRISMA protocol, which is especially designed for systematic reviews and meta-analytic studies, has been used.

In order to reach the intended studies, English data bases including Science Direct, PubMed, Scopus, Web of science, Medline along with Farsi data bases of SID, Magiran, Iranmedex, Irandoc, Medlib and Google Scholar search engine were searched within the interval of 1994 till 2017. The search was conducted drawing on key words of “Risk Factors; Accidental Falls; Aged; Hospitals; Systematic Review”. AND/OR were used to secure sensitivity of the search.

The inclusion criteria were studies which focused on risk factors for the elderly falling in hospitals. The exclusion criteria comprised studies which drew on nonrandom sampling methods, no reporting of the requisite data such as the size of the population or risk factors affecting the elderly fall, unacceptable quality of studies according to STROB Checklist, and lack of access to studies full-texts.

The international and standardized checklist of STROB was used to qualitatively evaluate the studies. This checklist comprised 22 sections covering different parts of a report. Based on this checklist, articles that had acquired the minimum score of 16 could enter the extraction stage within systematic review procedure.

Two researchers carried out data gleaning independently in order to minimize any error or bias in data gathering procedures. Data collection form was used based on the study aims. Researchers entered the elicited data to a checklist which encompassed the following: researcher’s name, title of the article, sample size, gender, age group, elderly falling risk factors, the year study conducted, and the country in which the study had been conducted. A third researcher examined the gleaned data by the two researchers looking for any inconsistencies or discrepancies. After completing the form, the resulting data were examined, summarized, and finally reported.

**Findings**

The initial search yielded 982 research papers. In the next stage, their titles were analyzed by two members of the team independently and 335 articles with content overlapping were eliminated from the study. Then, the articles abstracts were examined and 38 research papers for which their full texts were not available were removed from the study. Afterwards, the remaining articles were scrutinized in terms of the inclusion/exclusion criteria. After the meticulous and exhaustive reviewing of the articles, 590 articles that did not conform to our exclusion criteria were removed from the study. Finally, the remaining research papers were analyzed drawing upon STROB checklist which ended up in the exclusion of three more articles. In the end, 16 articles entered the systematic review process.

Characteristics of articles entered Systematic Review Process:

In the Case study Salgado, R (12) (1994) in Australia with 128 elderly (ccu word) result showed Risk Factors fall is Disorientation, Taking medicines Evidence of CVA and Poor performance in “Get-up-and-go” Test.

In the study Luukinen, H (16) (1995) in 145 elderly (Prolonged cares in hospitals / nursing homes) result showed Dizziness, evidence of CVA Consequences, high blood pressure, using walking aids, taking antidepressants, Lowering of muscular strength, Lowering of pulse in Art spot test, And lowering of walking pace.

In the Cass-control study Salgado ,R (17) (2003) in Australia with 88 elderly (ccu word) result showed Risk Factors fall is Disorientation, Taking psychiatric Drugs, Evidence of CVA and Poor performance in “Get-up-and-go” Test.

In the study Ishizuka, MA (18) (2005) in Brazil with 49 elderly (Geriatric care) result showed Risk Factors fall is Depression, history of referring to psychiatrist, Muscular Dystrophy.

In the Retrospective study Bollini, G (19) (2010) in Australia with 112 elderly (ccu word) result showed Risk Factors fall is High blood pressure, 15 or higher on Norton Scale, over 10 days of hospitalization
In the Retrospective study Freidman, SM (20) (2008) in USA with 212 elderly (ccu word) result showed Risk Factors fall is Presence of the elderly In nursing homes.

In the Case-control study Chen, YC (21) (2009) in Taiwan with 202 elderly (All Wards) result showed Risk Factors fall is Leg dystrophy, Insomnia, Blood pressure fall, History of Falling in the last year, Recent use of hypnotics.

In the study Bollini, G (19) (2010) in Italy with elderly (8 wards) result showed Risk Factors fall is Taking one or combined drugs.

In the Retrospective/ Case control study Chen, XL (22) (2010) in China with 438 elderly (Geriatric) result showed Risk Factors fall is living in dormitory/ nursing homes before hospital admission, requiring assistance for movement, cognition disorder, CVA, urinary incontinence and arthritis/ osteoporosis.

In the Retrospective study Church, S (23) (2011) in USA with 9625 elderly (Surgery Wards) result showed Risk Factors fall is Delirium 43%, Disability 34%, External factors 13%, Other factors 10%.

In the Case-control study Chen, X (24) (2011) in Australia with 399 elderly (Geriatric) result showed Risk Factors fall is History of Anemia, CVA, Ventricular Fibrillation, Over 5 weeks hospitalization.

In the Retrospective study Maxwell, CA (25) (2013) in USA with 101 elderly (Surgery Wards) result showed Risk Factors fall is Delirium 36 patients (44%) had cognition disorders and 62 patients (78%) had performance disorder prior to their fall.

In the Case-control study Chan, CH (26) (2013) in USA with 290 elderly (Psychiatry Wards) result showed Bipolar Disorder, Psychiatric disorders, Manic episode, History of health problems Or fall in last 6 months.

In the Cohort study Jorgensen, TSH (27) (2015) in Denmark with 28524 elderly (All Wards) result showed People with dementia, osteoporosis, CVA, depression, COPD, and Parkinson.

In the Retrospective study Li, IF (28) (2016) in Taiwan with 244 elderly (Clinical, Oncology, Surgery word) result showed Dizziness and Leg dystrophy, taking medications such as tranquilizers, sleeping pills, blood pressure drugs, hypoglycemic, and diuretic drugs.

In the Descriptive-cross sectional study Moura, LA (5) (2017) in Brazil with 99 elderly (Clinical, Surgery) result showed High blood pressure, taking insulin, long walks.

Discussion

Systematic review studies provide comprehensive information. This study is the first systematic review study to evaluate the Risk Factors of Elderly Falling in Public Hospitals. The findings of the present study can be summarized into two important categories of factors underlying the elderly falls in hospitals: individual factors (physical, mental, cognitive) and external factors. Following a review study in 2013 in Iran, it was found that more than 30% of people over 65 experience falling at least once a year. Lowering of consciousness, convulsion, sudden myocardial infarction, and environmental problems were among the influential risk factors for the elderly falling. In another review study on risk factors for the elderly falling in hospitals in Iran in 2013, the risk factors were mostly either individual or external. The results of another review study demonstrated that the major risk factors for the elderly fall comprised balance disorders, visual disorders, lowering of cognitive abilities especially problems of concentration and noticing along with other factors such as increasing of age, gender, dizziness, cardio-vascular diseases, dementia, depression, and taking medications.

In another study, the elderly people who used walking aids were more prone to falling than others since some of the elderly may not be able to use such aids properly. In the current study, it was mentioned that in studies by Luukinen and Aizen it had been demonstrated that the elderly who used walking aids (such as walkers, clubs, etc.) more often were more likely to fall in hospitals. Moreover, the relationship between taking drugs (especially Amphetamines, sedatives, antidepressants, diuretics, and cardio-vascular medications) and the elderly fall. It was also demonstrated that the elderly who take more than one drug are more likely to fall. It has also been claimed that antipsychotic medications, anti-arrhythmia, digoxin, diuretics, as well as tranquilizing medicines increase the risk of the elderly falling in hospitals.

Conclusion

In CCU, prolonged care, psychiatry, and geriatric wards in hospitals there are different factors contributing
to the elderly fall, depending on their physical and mental states. Thus, identifying and eliminating these risk factors can guarantee more safety for the elderly.

Conflict of Interest: There is no conflict of interest between authors.

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Source of Funding: Ilam University of Medical Sciences

Ethical Clearance: Ethics Code IR.MEDILAM.REC.1397.097

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20. Friedman S, Mendelson D, Bingham K, McCann R. Hazards of Hospitalization: Residence Prior to


Effect of Web-Based Early Diagnosis of Dental and Oral Diseases with Validity Level of Dentist Final Diagnosis in Public Health Center, Makassar, Indonesia

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¹Associate Professor, Department of Epidemiology, Faculty of Public Health, Universitas Muslim Indonesia, Makassar; ²Associate Professor, Departement of Information System, Faculty of Computer Science, Universitas Muslim Indonesia, Makassar

Abstract

Background. One of the organs of the body that is lacking attention is the teeth and mouth, whereas maintaining healthy teeth and mouth is very important, because the nerves of the teeth are related and directly affect the nerves of other organs. The development of information technology that is so advanced has entered the world of health, namely to detect early dental and oral diseases by using an expert system. Making an expert system is not to replace the expert itself but can be used as a very experienced assistant. There is a need to solve this problem so that people can know how to deal with diseases related to teeth and mouth. The various information supporting factors in this study by building an expert system with three methods are Certainty Factor (CF), Dempster Shafer (DS) and Bayes Theorem to diagnosis early dental and oral disease events and determine the effect of expert systems in increasing the validity level of dentist diagnosis. Material and Method The type of research used was an observational method in a cross-sectional study design. The sampling method uses total sampling. The study was conducted in September-January 2020. The number of sampling was 200 people. This research consists of three stages are the making of an application system, application testing, and the final stage is a statistical test. The data analysis technique used in this study is Chi-square and validity test. Results. The results of the analysis of the relationship between the value of accuracy with the validity of the doctor’s diagnosis found variable diagnosis doctor have p value (0.013), DS value, CF and Bayes Value are the same p (0,000) <p (0.05). Conclusion. Applications built using the CF, DS and Bayes Theorem methods can be used by users to detect dental disease early before making further examinations to a specialist. The test results given by the system are the same as the results given by experts (dentists). The system of early diagnosis of dental and oral diseases has a very significant effect on the level of validity of the dentist final diagnosis.

Keyword: System Expert, Dental and Oral Disease, Validity, CF, DS and Bayes, diagnosis doctor

Background

One of the organs of the body that is lacking attention is the teeth and mouth, whereas maintaining healthy teeth and mouth is very important, because the nerves of the teeth are related and directly affect the nerves of other organs.¹,² The development of information technology that is so advanced has entered the world of health are to detect early dental and oral diseases by using an expert system. Making an expert system is not to replace the expert itself but can be used as a very experienced assistant. In addition, lack of knowledge and limited sources of information cause low public awareness of efforts to prevent and even treat oral and dental diseases. Public understanding of dental and mouth disease is still relatively low so that many people still rely on the knowledge of an expert in this case doctors to be able to diagnose an illness, so it requires a long time and expensive costs. Tooth and mouth disease can cause pain and tooth loss. The high prevalence of dental and mouth disease is generally caused by various

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factors, including: factors of knowledge, attitudes and actions in maintaining dental health that is still low.\[^{3,4,5,6,7}\] In addition to expensive consultation fees, long queues and looming pain are also the reasons people are afraid to see a dentist.

There is a need to solve this problem so that people can know how to deal with diseases related to teeth and mouth. The various information supporting factors in this study by building an expert system with three methods are CF, DF and Bayes to diagnosis early dental and oral disease events and determine the effect of expert systems in increasing the validity level of dentist diagnosis. Based on the above background, the authors wish to discuss research on the early diagnosis of dental and oral diseases with the level of validity of the diagnosis of dentists in the Public Health Center Makassar, Indonesia.

**Material and Method**

The type of research used was an observational method in a cross-sectional study design. The sampling method uses total sampling. The study was conducted in September-January 2020. The number of sampling was 200 people. This research consists of three stages are the making of an application system, application testing, and the final stage is a statistical test. The data analysis technique used in this study is Chi-square and validity test.

**Results**

Data collection was carried out to obtain some information related to making an expert system application for diagnosis of dental and oral diseases are the form of symptom data and disease data. Data obtained during the data collection process was obtained from the results of patient interviews (200 people) with dentists, nurses, and supported by books, journals, and internet browsing related to dental and oral diseases. Data is then processed by the system so that it becomes input data and output data. The method in analyzing data using the algorithm is the CF, DS and Bayes methods.

1. **Design Form**

The screen will be used to log in by the user by entering a username and password. The design form of the login menu screen is as shown in Figures 1 and 2.
The user/admin screen has facilities for analyzing diseases. If the user as an admin, then has the facility to add user data or disease data, while the user is not allowed to add data. The shape of the design can be seen in Figure 3.
2. Relationship of Expert Diagnosis System with Validity Doctor’s diagnosis

**Table 1 Distribution of Respondent Characteristics by Gender, Age, Doctor Check Up, Occupation, Marital Status, Accuracy Value, DF, CS and Bayes**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency</th>
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<tr>
<td>Male</td>
<td>71</td>
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<tr>
<td>Age</td>
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<tr>
<td>5-15 years</td>
<td>47</td>
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<tr>
<td>16-26 years</td>
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<td>32,5</td>
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<td>1,5</td>
</tr>
<tr>
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<td></td>
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<tr>
<td>Doctor A</td>
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<td>49,5</td>
</tr>
<tr>
<td>Doctor B</td>
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<td>Occupation</td>
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<td>Marital status</td>
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<td>Married</td>
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<tr>
<td>Single</td>
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<td>58,5</td>
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<tr>
<td>Accuracy Value</td>
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<tr>
<td>Accurate</td>
<td>122</td>
<td>61</td>
</tr>
<tr>
<td>No Accurate</td>
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<td>39</td>
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<tr>
<td>DS value</td>
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<tr>
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<td>123</td>
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</tr>
<tr>
<td>Sure</td>
<td>74</td>
<td>37</td>
</tr>
<tr>
<td>Almost Sure</td>
<td>3</td>
<td>1,5</td>
</tr>
<tr>
<td>Nilai CF</td>
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<tr>
<td>Sure</td>
<td>131</td>
<td>65,5</td>
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<tr>
<td>Almost Sure</td>
<td>69</td>
<td>34,5</td>
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<tr>
<td>Bayes Value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sure</td>
<td>126</td>
<td>63</td>
</tr>
<tr>
<td>Almost Sure</td>
<td>74</td>
<td>37</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>100</td>
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</table>
### Table 2 Relationship of Accuracy Values with Doctor Diagnosis Validity

<table>
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<tr>
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<th>Accuracy Values with Doctor Diagnosis</th>
<th>Pvalue</th>
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</thead>
<tbody>
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<td></td>
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<td>No Accurace</td>
</tr>
<tr>
<td></td>
<td>Frequenci</td>
<td>Percent</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-15 years</td>
<td>30</td>
<td>24,6</td>
</tr>
<tr>
<td>16-26 years</td>
<td>42</td>
<td>34,4</td>
</tr>
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<td>27-37 years</td>
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<td>20,5</td>
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<tr>
<td>38-48 years</td>
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<td>49-59 years</td>
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<tr>
<td>Marital Status</td>
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<tr>
<td>Married</td>
<td>47</td>
<td>38,5</td>
</tr>
<tr>
<td>Single</td>
<td>75</td>
<td>61,5</td>
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<tr>
<td>Diagnosis Doctor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doctor A</td>
<td>69</td>
<td>56,5</td>
</tr>
<tr>
<td>Doctor B</td>
<td>53</td>
<td>43,5</td>
</tr>
<tr>
<td>DS Value</td>
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<td></td>
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<tr>
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<td>77,6</td>
</tr>
<tr>
<td>Sure</td>
<td>25</td>
<td>20,4</td>
</tr>
<tr>
<td>Almost Sure</td>
<td>0</td>
<td>0</td>
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<td>CF Value</td>
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<td>Sure</td>
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<tr>
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<td>79,5</td>
</tr>
<tr>
<td>Almost Sure</td>
<td>25</td>
<td>20,5</td>
</tr>
<tr>
<td>Total</td>
<td>122</td>
<td>100</td>
</tr>
</tbody>
</table>
Discussion

Expert system is one branch of artificial intelligence, which is a computerized application that tries to imitate the reasoning process of an expert in solving specific problems by making a decision. The basic of an expert system is how to transfer the knowledge which are owned by an expert in this case is dentist to a computer, and how to make decisions and draw conclusions based on that knowledge. [8], [9], [10], [11], [12]

As the times evolve, the field of dentistry has utilized technology in an effort to improve better services in diagnosing diseases, one of which is dental and oral disease. [13], [14] Teeth are one of the chewing organs that are very important in digestion process in the human body. [15], [16] If the tooth is disrupted, it will cause a variety of complaints and symptoms that can be fatal. Considering that experts and hours of practice are limited, so patients cannot consult with experts anytime and anywhere, we need an expert system that can replace the role of an expert. [17], [18]

The research results that will be discussed are the expert system designed using three methods, the number of diseases discussed in this study as many as 19 diseases with 61 symptoms observed, and the knowledge base is represented in the form of If-Then Rules. Each table of research results will be interpreted and given an accurate explanation.

Table 1 explains that the number of respondents who filled the expert system were 200 people with the most respondents, namely women (64.5%), ages 38-48 (11.5%), Doctor B (50.5%), not working (63.5%), unmarried (58.5%), Accuracy value (61%), DS Value (61.5%), CF Value (65.5%) and Bayes Value (63%). From the results of a trial of 200 cases of expert system diagnosis data with the doctor diagnosis results obtained in accordance with using the three methods. The test results can be used as a percentage that with expert knowledge that is obtained results above 60% of the truth value. This is different from the results of research conducted by Novi. AH (2014) which explains that the truth percentage of expert knowledge in diagnosing diseases is 100% using one method, the DF method. [19]

This is in accordance with DF theory which explains that a mathematical theory for proving hypotheses is based on belief functions and plausible reasoning, which is used to combine separate pieces of information (evidence) to calculate the likelihood of an event. The DF theory is based on two ideas namely the idea of obtaining a degree of trust from various subjective possibilities and the rules of the DF itself to combine the degree of trust based on the evidence obtained. DS theory is generally written in certain time intervals. [20]

Another study conducted by Jaenal Arifin using another method is CF explained that the level of trial results to determine the diagnosis of dental and oral diseases with a trial of 10 data resulted in a match rate of 9 data so that the accuracy rate was 90%. [21] Research conducted by Ingrid N, MY, etc. (2016) explains that the application of an expert system uses the CF method in early detection of dental disease before further examination to the expert physician showing the results that the results given by the system are the same as the results given by the system expert, is dentist. [22]

CF is a method to prove whether a fact is certain or uncertain in the metric form that is usually used in expert systems. CF was introduced by Short life Buchanan in making MYCIN. CF is the clinical parameter value given by MYCIN to show the magnitude of the trust. [23]

The advantage of the CF method is suitable for use in expert systems to measure whether something is certain or uncertain, calculations using this method in a single count can only process 2 data so that the accuracy of the data can be maintained. The disadvantages of the Certainty Factor method are: the general idea of modeling human uncertainty using numerical certainty factor methods is usually debated, this method can only process uncertainty or certainty in only 2 data only. Need to do several times the processing of data that is more than 2 pieces. [24]

Some of the research results conducted by previous researchers show that the method used is only one method and uses relatively little data. The data used not come from data directly from patients, while the research we used was using 3 methods at the same time. The use of three methods at the same time is used to see whether the results are different or not, but the results obtained are all the same percentage.

Table 2 explains the significant relationship between the value of accuracy with the validity of the doctor’s diagnosis results seen from the diagnosis doctor variable \( p (0.013) < p (0.05) \), DS, CF and Bayes Value each
having the same value is \( p (0.000) < p (0.05) \).

After seeing that there is a significant relationship between the diagnosis doctor variable and the expert system accuracy value then it proves there are similarities between the results of the 2 dentist examinations and the computer expert system. The similarity of the results of the diagnosis of the two doctors shows that the competency between one dentist and the other is the same so that in research it is proven that whatever or whoever is involved in the early diagnosis of the disease through an expert system, it will result in a high accuracy value.

Therefore, the expert system is designed to be able to imitate the expertise of an expert in answering questions and solving a problem. Expert system will provide a solution to a problem obtained from dialogue with users. With the help of an expert system, a person who is not an expert or expert can answer questions, solve problems and make decisions that are usually made by an expert. \[24\]

Knowledge stored on a computer is called a knowledge base. There are 2 types of knowledge are facts and procedures. One feature that must be possessed by an expert system is the ability to reason. If the skills are stored as a knowledge base and the program is able to access the database, then the computer must be programmed to make inferences. This inference process is packaged in the form of an inference engine. Most commercial expert systems are made in the form of rule-based systems, where the knowledge is stored in the form of rules. The rule is usually in the form of IF-THEN. Another feature of the expert system is the ability to recommend. This ability distinguishes expert systems from conventional systems. \[7\]

**Conclusion**

Applications built using the Certainty Factor, Dempster Shafer and Bayes methods can be used by users to detect dental disease early before making further examinations to a specialist. The test results given by the system are the same as the results given by experts (dentists). The system of early diagnosis of dental and oral diseases has a very significant effect (\( p 0.00 \)) on the level of validity of the dentist final diagnosis.

**Ethical Considerations:** Ethical clearance was obtained from Universitas Muslim Indonesia; with number “320/A/KEPK- UMI/VIII/2019. Just before the interview, written (or thumb impression) consent was obtained from each participant in Universitas Muslim Indonesia guidelines.

**Conflicts of Interest:** The authors alone are responsible for the views expressed in this article and they do not necessarily represent the views, decisions, or policies of the institutions with which they are affiliated.

**References**


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oral medicine, 2003. PMPH, USA.


Association between Intelligence Level and Handicap Degree in Epilepsy Patients

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¹Department of Neurology, Faculty of Medicine, Universitas Airlangga, Surabaya (60131), Indonesia

Abstract

Background: Epilepsy correlates with high limitation value, disability and function loss which can give negative impact to patient’s quality of life. This negative impact correlates with either epileptic seizures (motoric, sensory and behavior) or effects of anti-epileptic drugs that can cause cognitive impairment, which in this case is intelligence aspect with handicap in epilepsy patients. We determined correlation between intelligence level and handicap degree in epilepsy patients.

Methods: The research was conducted in patients of Neurological Department of Dr. Soetomo General Hospital, Surabaya, Indonesia, who met inclusion and exclusion criteria, consecutively from July 2013-December 2013. Intelligence test was conducted using Standard Progressive Matrices (SPM) test, while test on handicap degree was conducted using Subjective Handicap Epilepsy (SHE) test.

Results: There were 40 research subjects (19 females and 21 males) with average age of 30.9±9.6. The average score of SHE was 70.08±16.69, while the average score of SPM was 88.53±10.34. There was a weak positive correlation and statistically significant correlation between SPM and SHE scores (r = 0.345 and p = 0.029).

Conclusion: There was a correlation between intelligence level and handicap degree in epilepsy patients.

Keywords: Epilepsy, Handicap, Intelligence

Introduction

Epilepsy is a chronic neurological disease marked by unprovoked epileptic seizures that occurs more than once a year. According to PERDOSSI (2012) there is a high prevalence of epilepsy in developing countries, with number reaching 114 per 100.000 population per year compared to that in development countries. An estimated 250.000 people per year in Indonesia suffer from epilepsy¹.

Epilepsy is caused by curse, magic, possession, mental disorders, and it is often regarded as a disease transmitted through saliva (Harsono, 2012). Limited medical personnel, service facilities, funding and society’s ability decrease epilepsy prevention. The disease can be controlled with medication and surgery².

Epilepsy can also deal with intelligence impairment, with prevalence of its occurrence being estimated to be 14% until 44% that has been reported by various studies conducted in United Kingdom. Furthermore, epilepsy patient with intelligence impairment usually has health problems, including fractures, trauma and side effects of anti-epileptic drugs³. Epilepsy also strongly correlates with intelligence level, which has important roles in handicap. The previous research has explained the correlation between intelligence level and handicap degree, thus this study aims to improve intelligence quality among disabled.

Method

The research is using cross sectional design. The study used sample of all epilepsy patients who visited Outpatient Neurological Clinic of Dr. Soetomo General Hospital, Surabaya, Indonesia from July to December of 2014. The research was conducted at the Outpatient Neurological Clinic of Mental Health Science, Dr. Soetomo General Hospital, Surabaya, Indonesia, for four months⁴.
The following are inclusion criteria that must be fulfilled in the research is epilepsy patients aged 13 – 55 years old, having minimum education of elementary school and can read and write. Being diagnosed with epilepsy at least for 1 year, patients are willing to participate in the research (informed consent). The research employed consecutive sampling method. The research variables were intelligence level as independent variable, handicap degree as dependent variable, and sickness duration, types of epileptic seizure, as well as types of therapy as confounding variables. The study protocol was approved by the ethics committees of Dr. Soetomo Teaching Hospital (Surabaya, Indonesia).

The research began with patients declaring their willingness to become the subjects of the study by signing informed consent. The sampling process continued by conducting anamnesis, physical and neurological examination based on inclusion and exclusion criteria. Afterwards, the researchers conducted handicap measurement based on SHE score issued by PPDS in Epilepsy unit. Intelligent test was then performed by a psychologist at Psychology Unit of Outpatient Clinic of Mental Health Science, before the researchers recorded all eligible samples and necessary clinical data. The records were collected to make data tabulation and statistical analysis. The study employed Spearman test to analyze the data since the data were not normally distributed; the results have strong positive correlation and statistically significant (r=0.697 and p=0.025). Statistic calculation was conducted using SPSS software (SPSS, Inc., Chicago, IL).

Results

The results of data processing on distribution of clinical data that includes types of seizure, seizure frequency and types of therapy are shown in Table 1, while frequency distribution of sickness duration, SHE and SPM scores are presented in Table 2. Based on the results of analysis on frequency distribution of seizure types, Table 1 showed 15 subjects with focal seizure (37.5%) and 25 subjects with general seizure (62.5%). On the other hand, frequency distribution of seizure frequency showed 14 subjects (35%) with frequency <1x/year and 26 subjects (65%) with frequency ≥1x/year. Frequency distribution of types of therapy showed 21 subjects were treated with monotherapy (52.5%) and 19 subjects with polytherapy (47.5%). The average SHE score was 70.08±16.69, while the average SPM score was 88.53±10.34.

The results of correlation analysis were divided into six data correlation analyses using Spearman test that are shown in Table 3. The results of data processing on correlation between intelligence level (SPM score) and handicap degree (SHE score) based on educational level showed a moderate positive correlation, but it was not statistically significant, between SHE and SPM scores in the sample group of elementary-junior high school graduates, with correlation coefficient (r) of 0.478 and p=0.137. Meanwhile for the sample group of senior high school-university graduates, there was a weak positive correlation between SHE and SPM scores (r=0.128 and p=0.508).

The results of data processing on correlation between intelligence level (SPM score) and handicap score (SHE score) based on the subjects’ occupation showed a powerful positive correlation between SPM and SHE scores in the sample group of students-college students, with correlation coefficient of (r)=0.886 and p=0.019, and the correlation was statistically significant. In the sample group of employed subjects, there was no correlation between SPM and SHE scores with correlation coefficient (r)=0.165 and it was not statistically significant with p=0.449. Meanwhile in the sample group of unemployed subjects, there was a moderate positive correlation, but it was not statistically significant, between SPM and SHE scores with r=0.425 and p=0.221.

The results of data processing on correlation between intelligence level (SPM score) and handicap degree (SHE score) based on seizure types showed a weak positive correlation between SPM and SHE scores in the subjects with focal seizures, with correlation coefficient value (r)=0.216, and it was not statistically significant with p=0.440. Meanwhile in the sample group of general seizure, there was a moderate positive correlation between SPM and SHE scores with r=0.419, and it was statistically significant with p=0.037.

The results of data processing on correlation between intelligence level (SPM score) and handicap degree (SHE score) based on seizure frequency showed a weak positive correlation between SPM and SHE scores in the sample group with frequency <1x/year, with r=0.347, and it was not statistically significant with p=0.224. Meanwhile, there was a moderate positive correlation between SPM and SHE scores in the sample group with frequency ≥1x/year, with r=0.505, and it was statistically significant with p = 0.008.
The results of data processing on correlation between intelligence level (SPM score) and handicap degree (SHE score) based on types of therapy showed no correlation between SPM and SHE scores in the sample group with monotherapy, with $r=0.098$ and $p=0.673$. Meanwhile, there was a moderate positive correlation between SPM and SHE scores in the sample group with polytherapy, with $r=0.574$, and it was statistically significant with $p=0.010$. From the results of overall analysis, there was a weak correlation between SPM and SHE scores with $r=0.345$, and it was statistically significant with $p=0.029$.

**Table 1. Clinical data by types of seizure, seizure frequency, types of therapy**

<table>
<thead>
<tr>
<th>Clinical Data Distribution</th>
<th>Value</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Types of seizure</td>
<td></td>
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<tr>
<td>Focal seizure</td>
<td>15</td>
<td>37.5 %</td>
</tr>
<tr>
<td>General seizure</td>
<td>25</td>
<td>62.5 %</td>
</tr>
<tr>
<td>Seizure frequency</td>
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<td></td>
</tr>
<tr>
<td>&lt;1x/year</td>
<td>14</td>
<td>35%</td>
</tr>
<tr>
<td>≥1x/year</td>
<td>26</td>
<td>65%</td>
</tr>
<tr>
<td>Types of therapy</td>
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<td></td>
</tr>
<tr>
<td>Monotherapy</td>
<td>21</td>
<td>52.5%</td>
</tr>
<tr>
<td>Polytherapy</td>
<td>19</td>
<td>47.5%</td>
</tr>
</tbody>
</table>

**Table 2. Clinical data by sickness duration, SHE score and SPM score**

<table>
<thead>
<tr>
<th>Clinical Data Distribution</th>
<th>Mean ± SB</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sickness duration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sickness duration (year)</td>
<td>11.78 ± 8.74</td>
<td>1-41</td>
</tr>
<tr>
<td>Handicap degree (SHE score)</td>
<td>70.08 ± 16.69</td>
<td>30-99</td>
</tr>
<tr>
<td>Intelligence level (SPM score)</td>
<td>88.53 ± 10.34</td>
<td>70 - 112</td>
</tr>
</tbody>
</table>

**Table 3. Results of correlation assessment**

<table>
<thead>
<tr>
<th>Variable(s)</th>
<th>Correlation coefficient ($r$)</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation between intelligence level (SPM score) and handicap degree (SHE score) based on education level</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPM score vs SHE score (Elementary-Junior High School)</td>
<td>0.478</td>
<td>0.137</td>
</tr>
<tr>
<td>SPM score vs SHE score (Senior High School-University)</td>
<td>0.128</td>
<td>0.508</td>
</tr>
</tbody>
</table>
Discussion

Based on the results of data processing on epilepsy patients’ education level, there were 11 subjects (27.5%) graduated from elementary-junior high school, while there were 29 subjects (72.5%) graduated from senior high school-university. The results of data processing on the patients’ occupation showed 6 students and college students (6%), 24 subjects were employed (60%) and 10 subjects were unemployed (25%). Thomas SV (2005) explained that most subjects could complete their secondary education and have a job.

Types of anti-epileptic drugs (AEDs) administered to the research subjects were old AEDs, including carbamazepine, valproic acid, phenytoin and clobazam. The average dose used in the research, either monotherapy or combination of polytherapy, was still in the therapeutic range. The research found that monotherapy was more frequently used than polytherapy. Thus, this finding was consistent with the previous studies which stated that monotherapy was more frequently used than polytherapy for epilepsy patients, including the studies conducted by Cantey (5:1 ratio, n = 145) and Sejvar (3:1 ratio, n = 112).

The results of data correlation processing showed a powerful positive correlation and statistically significant correlation between SPM and SHE scores in the sample group of students and college students. However, this finding cannot be compared with findings from other studies since there is no journal with similar discussion. The present study divided occupational characteristics into two groups, namely students-college students and employed-unemployed. Thus, there was a possibility that students and college students have a more homogenous activity compared to employed subjects.
of employed subjects is more heterogenous because of varied types of occupation) 9.

There was a moderate positive correlation between SPM and SHE scores found in the group of subjects with seizure frequency of ≥1x/year (r=0.505 and p=0.008). This finding was consistent with studies conducted by Hawari I et al. (p=0.009) and Piperidou et al. (p=0.001). The finding of the present study is supported by a statement found in literatures that stated that the greater the physical and chronic health problems, the seizure will be more frequently occurred that can worsen epilepsy patients’ handicap. Research conducted by Dodril et al. also indicated that adult patients who suffer from episodes of general tonic clonic seizure more than 100 times tended to have worse intellectual, neuropsychological, psychosocial and emotional functioning than patients with fewer episodes of seizure 10.

Well-controlled seizure will maintain epilepsy patients’ cognitive function. Anti-epileptic drugs can control the work and also provide protection function that exceeds its side effects, they are: 1) by reducing seizure activity, where recurrent seizures can trigger neuronal damage and disrupt brain formation and circuits, 2) modulate neurotransmitters effects, reducing excitociticy by decreasing glutamate release, 3) inhibiting Ca²⁺-mediated cellular function and Ca²⁺- depended depolarization, 4) as a free radical scavenger, and 5) pleiotropic effects. The results of data correlation processing showed a moderate positive correlation (r=0.574 and p=0.010) between SPM and SHE scores in the subjects with polytherapy. This finding was consistent with studies conducted by Hawari I et al. (p=0.006) and Piperidou et al. (p<0.001)

Polytherapy have a relatively bigger effect on cognitive function compared to monotherapy, regardless the types of anti-epileptic drugs used in its combination. Combination of anti-epileptic drugs will worsen its cognitive side effects that can induce the occurrence of more serious cognitive impairments if the drugs are administered in the same time 11.

The research found a weak positive correlation between SPM and SHE scores (r=0.345), and it was statistically significant (p=0.029). This finding was consistent with a research conducted by Sabaz with p=0.000, as the finding stated that child epilepsy patients with intellectual impairment had a larger handicap (worse quality of life) compared to those with normal IQ. Gulhoto et al. in Brazil also stated that adolescents with focal seizures had a lower cognitive ability compared to normal adolescents. Epilepsy patients with intellectual impairment have higher emotional, behavioral and cognitive problems, with difficulty in socializing and having achievements, particularly in schoolchildren. The direction of a positive relation indicates that if SPM score increases, the SHE score also increases (smaller handicap) 12.

Although academic problems are the result of multifactorial, there is still a need for a more specific educational approach in order to improve the performance of these adolescents, in addition to helping them eliminate the stigma associated with epileptic seizures in society 13. Therefore, statistical analysis is true if the research hypothesis states that there is a correlation between intelligence level (SPM score) and handicap degree (SHE score) in epilepsy patients 14. The correlation can also occur due to effects of polytherapy, more seizure frequency towards handicap and worsening intelligence. Therefore, the results of this study are expected to be more clinically focused and precise in giving therapy for epilepsy patients by optimizing the monotherapy use of anti-epileptic drugs and more selective combination therapy.

**Conclusion**

There was a correlation between intelligence level and handicap degree in epilepsy patients.

**Ethical Clearance:** This research process involves participants in the survey using a questionnaire that was accordant with the ethical research principle based on the regulation of research ethic committee. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, nonmaleficence, and justice.

**Conflict of Interest:** The author reports no conflict of interest of this work.

**Source of Funding:** This study is done with individual funding.

**References**

Effect of Glutamine Before Gets Cisplatin on Aif and Bcl-2 in the Evidence of Apoptosis Cell Tubulus Proximal in Rats Kidney of *Rattus Norvegicus* Strain Wistar

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¹Department of Pathology Anatomy, ²Department of Pharmacology, Faculty of Medicine Universitas Airlangga Dr. Soetomo Teaching Hospital Surabaya Indonesia, ³Department of Internal Medicine Universitas Airlangga Haji Hospital Surabaya Indonesia, ⁴Department of Clinical Pathology, Faculty of Medicine Universitas Airlangga Dr. Soetomo Teaching Hospital Surabaya Indonesia

Abstract

Background: Increased incidence of cancer in the world also increases the use of chemotherapy agents. Cisplatin is one of the most effective chemotherapy agents, often used for the treatment of various solid tumors. The therapeutic effect of cisplatin significantly increased with increasing dosage.

Aim: The aim of the study was to investigate the role of glutamine in the prevention of apoptosis in proximal renal tubular epithelial cells administered by cisplatin chemotherapy, via an intrinsic caspase-independent apoptotic (AIF).

Methods: The study was enrolled thirty male Wistar rats. The rats were divided into three groups of treatments, group A (negative control), group B has given intraperitoneal cisplatin dose of 20 mg/KgBB, group C administered the intravenous injection of glutamine 100 mg/KgBB for 7 days.

Results: Analyzed ANOVA, mean expression of BCL-2 group B = 56.80 ± 4.39 than group A (control) = 63.7 ± 5.53 decreased not significant (p = 0.06), and mean expression of BCL-2 group C = 113.30 ± 8.92 increase significant compared to group A = 63.7 ± 5.53 (p = 0.000001) and group B = 56.80 ± 4.39 (p = 0.000001). The average AIF expression group C = 236.20 ± 17.58 decreased significantly compared to group B = 309.50 ± 8.08 (p = 0.000001). The average number of group apoptosis C = 151.80 ± 21.87 decreased significantly compared to group B = 255.20 ± 27.82 (p = 0.000001).

Conclusion: Glutamine can inhibit the expression of AIF, increase BCL-2 expression, and decrease apoptosis of epithelial cells of the renal proximal tubules given cisplatin.

Keywords: Glutamine, Cisplatin, Acute Kidney Injury

Introduction

Cancer is the second leading cause of death that contributing 13% of deaths from 22% of deaths from non-communicable diseases in the world¹. It is estimated that at least 22.2 million new cases will be diagnosed annually worldwide by 2030 with the estimated deaths from cancer worldwide will rise through 13.1 million deaths in 2030². Ministry of Health Indonesia in 2007 reported that cancer is the leading cause of death number 7 in Indonesia with cancer prevalence 4.3 per 1000 population.

Cisplatin (cis-diammine-dichloroplatinum II) is one of the most effective chemotherapy agents, often used for...
the treatment of various solid human tumors including testicular and ovarian cancers\textsuperscript{3}. This is evidenced by the use of cisplatin as a standard component in chemotherapy regimens in various types of cancer, such as head and neck cancer, ovarian cancer, testicular cancer, esophageal cancer, bladder cancer, cervical cancer, and non-small cell lung cancer\textsuperscript{4}.

The therapeutic effect of cisplatin significantly increases with increasing dosage\textsuperscript{5}. However, increased doses of cisplatin in clinical use, often limited by adverse events, such as nephrotoxicity and neurotoxicity, are side effects that limit the dose of cisplatin\textsuperscript{6}. The main side effect that limiting the dose of cisplatin is the effect of nephrotoxicity\textsuperscript{7}.

There are various percentages of nephrotoxicity effects of cisplatin, but the most serious and most frequent effect is acute kidney injury (AKI) occurring in 25-35\% of patients\textsuperscript{8}. Conceptually AKI is a rapid decline (in hours to weeks) of a generally reversible glomerular filtration rate (LFG), followed by renal failure to excrete residual nitrogen metabolism, with/without disturbance of fluid and electrolyte balance\textsuperscript{9}.

The diagnostic criteria for AKI according to acute kidney injury network (AKIN) are: abrupt decrease in renal function (within 48 hours) characterized by elevated serum creatinine levels $> 0.3$ mg/dl (26.4 μmol/l) or serum creatinine more than 1.5 times ($> 50\%$) when compared with previous levels or decreased urine output to less than 0.5 cc/h for more than 6 hours\textsuperscript{10}. The nephrotoxic effects of cisplatin most commonly occur after 10 days of cisplatin administration\textsuperscript{11}. Over the past few years, renal tubular epithelial cell apoptosis has been the focus of investigation on the mechanism of nephrotoxicity of cisplatin. There are several apoptotic pathways involved, including extrinsic pathways mediated by death receptor, mitochondrial-centered intrinsic pathways, and ER pathway reticulum (ER) stress\textsuperscript{12}.

Increased incidence of cancer in the world also increases the use of various chemotherapy agents, including cisplatin. The widespread use of cisplatin increases the side effects of cisplatin use, including the nephrotoxic effects of cisplatin. Acute Kidney Injury (AKI), caused by renal cell apoptosis. Glutamine is the most abundant amino acid found in the blood and in the storage of free amino acids from the body. One of the important characteristics of glutamine is that glutamine has a beneficial effect on critical illness, as it can increase the expression of heat shock protein (HSP)\textsuperscript{13}. HSP is a group of essential proteins for cell endurance under stress conditions\textsuperscript{14}. There is evidence that glutamine can increase HSP-70 and HSP-72 expression in pulmonary and epithelial cell macrophages\textsuperscript{15}. Increased expression of HSP-72 has the ability of antiapoptosis by stimulating Bcl 2, Bcl-XL and inhibiting damage to the mitochondrial membrane\textsuperscript{16}. Therefore, this study will examine the effect of glutamine injection as an antiapoptosis of proximal renal tubular cells treated with cisplatin chemotherapy.

**Method**

This research was a laboratory experimental research with “Randomized Post-test only control group design” design that used thirty male Wistar rats. Rats were divided into three treatment groups, group A (negative control), group B has given intraperitoneal cisplatin injection of a single dose of 20 mg/KgBB, group C given intravenous injection glutamine 100 mg/KgBB for 7 days and at day 7 were given intraperitoneal cisplatin dose of 20 mg/KgBB. After 72 hours of cisplatin injection, renal mouse tissue was processed immunohistochemically, to observe the number of proximal renal tubular cells expressing Bcl-2, AIF, and apoptotic cell counts.

The experimental animals used in this study were obtained from the Faculty of Veterinary Medicine of Airlangga University. Selection of sample for grouping and giving treatment using simple random sampling, with inclusion criteria is *Rattus novergicus* strain Wistar rats, healthy male rats, healthy-looking active, 2.5-3 months old, 150-200 gram weight.

The sample size in this study was determined based on Federer’s formula, with dropout correction (0.1), resulting in a total sample of 30 mice with details of 10 rats for each treatment group. Animal treatment was performed for 7 days, then examination of kidney preparation on the 10th day was the number of cells expressing BCL-2, the number of cells expressing Apoptotic Inducing Factor AIF and the number of apoptotic cells in male rats proximal tubule rats *Rattus novergicus* wistar strain, with a 1000X light microscope, positive rectal proximal tubular cells were calculated (located in the renal cortical region, characterized by cylindrical tube lumen), positive cell counts in 20 viewing field (HPF) in each sample.
The duration of Injectable Glutamine Intra-Vein (IGIV) administration was 7 days, because based on previous studies glutamine will increase HSP after 7 days of IGIV. Observation time at day 10 or 72 hours after intraperitoneal cisplatin injection (on day 7) because apoptosis in proximal renal tubular cells was apparent after 72 hours of cisplatin administration. Renal kidney tissue is processed immunohistochemically, to observe the number of proximal renal tubular cells expressing Bel-2, AIF, and apoptotic cell count. Data were analyzed with SPSS 17, ANOVA analysis followed by Post Hoc Tukey test, with p-value <0.05.

Results

Based on the results obtained the analysis of glutamine inhibit apoptosis proximal tubule renal cells. Increased HSP70 affects survival associated with the ability to inhibit apoptosis. Glutamine supplementation enhances HSP expression. It was concluded that in the 72-hour observation after cisplatin administration, glutamine could inhibit apoptosis of proximal renal tubule cells significantly, and was directly proportional to the glutamine effect that inhibited the expression of AIF.

The homogeneity test shown in Table 1 was performed by using one-way ANOVA test, indicating that there was no significant difference between body weight before treatment and weight after treatment (sig >0.05). Here is table 1.

Table 1: Homogeneity Test Based on Weight Loss

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight before treatment</td>
<td>1.486</td>
<td>0.222*</td>
</tr>
<tr>
<td>Weight after treatment</td>
<td>0.941</td>
<td>0.449*</td>
</tr>
</tbody>
</table>

From that table, it can suggests that animal weight data try to have a homogeneous variation. In this study, it appears that changes in epithelial cells of proximal renal tubules occur after 72 hours of observation.

Immunohistochemical renal tissue examination results showed that the control group’s renal tissue showed an average amount of BCL-2 expression of 63.7 ± 5.538752. Giving cisplatin (Group B) in animals tries to decrease mean expression BCL-2 is not significant (p = 0.066028) after 72 hours to 56.80 ± 4.391912. Administration of cisplatin and glutamine (group C) in the experimental animals significantly increased the mean expression of BCL-2 to group A (p = 0.000001) and group B (p = 0.000001) after 72 hours to 113.30 ± 8.920015. Administration of cisplatin and glutamine (Group C), BCL-2 expression was increased when compared to the control group (Group A) and the cisplatin-treated group (Group B). Addition of cisplatin (Group B) did not significantly decrease BCL-2 expression compared to the control group (Group A). Histologic representation of BCL-2 expression with Immunohistochemical staining, using Olympus BX 50 magnification 1000x.

The results of immunohistochemical renal tissue examination of the average AIF group A (control) expression amounted to 48.4 ± 3.134042. Giving cisplatin (Group B) in animals tries to significantly increase the average of AIF expression (p = 0.000001) after 72 hours to 309.50 ± 8.086340. However, administration of cisplatin and glutamine in group C in animals tended to decrease mean AIF expression significantly against group B (p = 0.000001) after 72 hours to 236.20 ± 17.586611. It can be concluded that in the administration of cisplatin and glutamine (Group C), the expression of AIF decreased significantly compared with the group given cisplatin (Group B). A histologic representation of AIF expression with immunohistochemical staining using Olympus BX 50 1000x magnification microscope.

The results of the renal tissue examination with apoptosis detection kit In Situ Cell Death Detection Kit, POD showed that the group A (control) kidney tissue showed an average number of cell apoptosis of 47.40 ± 18.572381. Giving cisplatin (Group B) in animals tries to significantly increase cell apoptosis averages (p = 0.000001) after 72 hours to 255.20 ± 27.828043. However, the administration of cisplatin and glutamine in group C in animals tended to decrease the mean cell apoptosis significantly against group B (p = 0.000001) after 72 hours to 151.80 ± 21.877436. It can be concluded that in the administration of cisplatin and glutamine (Group C), rectal proximal renal tube apoptosis decreased significantly compared to the cisplatin group (Group B). Histologic features of rectal proximal tubular apoptosis with detection kit In Situ Cell Death Detection Kit, POD, using Olympus BX 50 1000x magnification.

The results of data analysis obtained showed that cisplatin and glutamine can increase BCL-2 expression, so BCL-2/Bax ratio can be increased which will inhibit
the leakage of mitochondrial membrane, and inhibit the release of apoptogenic factors (cytochrome c, apoptosis inducing factor (AIF), Second Mitochondria-Derived Activator of Caspase (SMAC), and others)\textsuperscript{12}. Similar results were also obtained from previous studies which concluded that HSP-72 expression enhancement has anti-apoptotic ability by stimulating Bel 2, Bel-XL and inhibiting damage to mitochondrial membranes\textsuperscript{16}.

In other side cisplatin (cis-diammine-dichloroplatinum II) is one of the most effective chemotherapy agents, often used as chemotherapeutic reagents of various solid human tumors including testicular and ovarian cancers\textsuperscript{3}. This is evidenced by the use of cisplatin as a standard component in chemotherapy regimens in various types of cancer\textsuperscript{4}.

The therapeutic effect of cisplatin significantly increases with increasing dosage\textsuperscript{5}. However, increased doses of cisplatin in clinical use are often limited by adverse events, such as nephrotoxicity and neurotoxicity, which are side effects that limit the dose of the cisplatin\textsuperscript{6}. Giving glutamine to malignancy is still controversial. There are groups who argue that glutamine is the main food needed for tumor cell metabolism, so the administration of glutamine can enlarge the tumor and resistant to chemotherapy\textsuperscript{17}. Yet another group believes that glutamine intake is necessary as a nutritional intake of cancer and has antioxidant effects by increasing Glutathione (GSH) that can inhibit tumor growth otherwise it is known to reduce the side effects of chemotherapy agents Doxorubicin\textsuperscript{18}. Previous studies proving cisplatin may increase AIF expression in the renal proximal tubular cytosol, which then AIF out of the mitochondria, will accumulate in the cell nucleus that will induce apoptosis through the caspase-independent pathway\textsuperscript{19}.

Hsp 72 renal epithelial cells may decrease mitochondrial membrane injury by Hsp 72 interaction with BCL-2. Furthermore, the potential of L-glutamine to HSP 72 is associated with increased intestinal epithelial resistance to apoptotic injury, and the reduction of HSP 72 may be associated with increased caspase activity in glutamine deficiency. Thus, it is hoped that the administration of glutamine would be useful in increasing heat shock protein (HSP) 72 and BCL2 which ultimately decrease apoptosis of renal proximal tubule cells due to cisplatin.

Glutamine administration inhibits the expression of AIF so it can inhibit the occurrence of apoptosis. This result is not in accordance with previous research, the resistance of AIF expression is not due to increased expression of BCL-2, this indicates if the effect of cisplatin on decreasing expression of BCL-2 is very low. Therefore, observation for 72 hours after cisplatin administration, glutamine administration can inhibit AIF expression significantly. Administration of cisplatin may increase apoptosis of proximal renal tubular cells that are mechanically nephrotoxic of cisplatin\textsuperscript{(15)}. Apoptosis due to cisplatin administration occurs through increased AIF expression. AIF that comes out of the mitochondria, will accumulate in the cell nucleus that will induce apoptosis through the caspase independent pathway.

**Conclusion**

Based on the results of the study it can be concluded that glutamine can increase BCL-2 expression in epithelial cells of the renal proximal tubules given cisplatin.

**Conflict of Interest:** Nil

**Source of Funding:** Self

**Ethical Clearance:** This study was approved by Ethical Commission of Health Research Faculty of Medicine University of Airlangga.

**References**


Association between Soluble Contents CD40 Ligand (sCD40L) and Acute Coronary Syndrome (ACS)

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Abstract

Background: Acute coronary syndrome (ACS) is a manifestation of coronary heart disease (CHD), which the leading cause of death in Indonesia. CD40 ligand (CD40L) stored in alpha platelet granules will be rapidly transferred to the surface when the platelets are activated and subsequently released from the surface as a soluble CD40 ligand (sCD40L). Soluble CD40 ligand (sCD40L) acts as a bridge between the inflammatory process, atherosclerosis, and thrombosis. This study aims to study the relationship between sCD40L levels and the incidence of ACS in patients with chest pain in Dr.Soetomo General Hospital, Surabaya.

Method: Research subjects were 40 patients with chest pain who came to Emergency Room at Dr.Soetomo General Hospital, Surabaya. The patients were grouped based on the diagnosis of ACS and non-ACS with the electrocardiogram and troponin T. Serum levels of patients that examined by sCD40L with enamine-linked immunosorbent assay (ELISA) method from Quantikine®.

Results: Twenty-six (65%) were diagnosed with ACS and 14 (35%) were non ACS. The diagnosis of ACS includes ST-segment elevation myocardial infarction (STEMI), non-ST-segment elevation myocardial infarction (NSTEMI), and unstable angina (UA), with the highest proportion being STEMI at 15 (57%). Spearman correlation test of sCD40L level with ACS incidence got correlation coefficient rho (ρ) = 0.52 (p = 0.001).

Conclusion: There was a moderate positive correlation between sCD40L levels and ACS incidence in patients with chest pain.

Keywords: CD40L, Acute Coronary Syndrome, Chest Pain, Unstable Angina

Introduction

Cardiovascular disease is a global health problem responsible for 30% of death globally. Data in 2005 suggested that 17 million deaths from a total of 58 million deaths worldwide were caused by cardiovascular disease ¹. The American Heart Association (AHA) mentions that 71.3 million Americans suffer from the cardiovascular disease in 2003. Cardiovascular disease accounted for nearly 1 million deaths in 2003 with 53% of those caused by coronary heart disease (CHD) ².

The mortality rate due to cardiovascular disease in Indonesia also increases annually, reaching almost 30% in 2004 compared to only 5% in 1975. Data from the Indonesian National Health Survey conducted by the Ministry of Health of the Republic of Indonesia stated that cardiovascular disease is the leading cause of death in Indonesia with CHD as the main cause by 26.4% ³.

The manifestations of CHD are the occurrence of acute coronary syndromes (ACS), which include unstable angina/UA (angle) and acute myocardial infarction (AMI). The data from the Directorate General of Pharmaceutical and Medical Devices of the Ministry of Health of the Republic of Indonesia in 2006 stated that ACS caused a huge number of hospitalizations and a major and most frequent cause of death for CHD.

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The high rate of ACS death causes the need for a proper understanding of the pathogenesis disease, thus helping to find a quick and accurate examination for diagnosis. Biological markers currently used for diagnosis of molecules released after the occurrence of myocardial necrosis, such as troponin and natriuretic peptides. The markers are clinically useful, but they have not been able to describe the pathogenesis of the early phase of atherothrombosis. An alternate marker is needed that can not only help diagnosis but may also provide an understanding of the pathogenesis of ACS.

The concepts put forward today are that inflammatory markers, endothelial dysfunction, and platelet activation that can be used to identify disease activity even before myocardial necrosis occurs. One of the concepts underlying the mechanism of atherothrombotic pathogenesis involves the interaction between CD40 and CD40 ligand (CD40L). CD40 is a transmembrane protein receptor that belongs to the group of superfamily tumor necrosis factor (TNF). CD40L is stored in the alpha platelet granule and is rapidly transferred to the surface at which platelets are activated. CD40L that has been expressed on the surface will soon be broken down and released from the surface of platelets as soluble CD40 ligand (sCD40L).

Until now the role of sCD40L in the process of ACS has not been fully understood. Several studies have shown that CD40 and CD40L are one of the mediators of vascular inflammation and act as a bridge between the inflammatory process, atherosclerosis, and thrombosis. Research on the sCD40L level and its relation to ACS incidence have not been performed, so that this study aims to know the relationship between sCD40L level with ACS incidence in Dr. Soetomo General Hospital, Surabaya.

**Method**

This research uses analytic observational research design with the cross-sectional design. The study was conducted at Emergency Room of Dr. Soetomo General Hospital, Surabaya, and Installation of Clinical Pathology Faculty of Medicine Universitas Airlangga/Dr. Soetomo General Hospital Surabaya. This research implemented in April to June 2013. All patients who become the sample of the study must meet the inclusion criteria, among others; patients with chest pain, aged ≥30 years, have complete medical records. While for exclusion criteria, among others; patients had sepsis, acute stroke, until serum creatinine>2 mg/dl.

The laboratory procedure in this study was started by examining the sCD40L level using the enzyme-linked immunosorbent assay (ELISA) method of Quantikine®. Then the inspection principle of sCD40L was an enzyme-labeled enzyme by the sandwich. The microplate coating was coated with polyclonal antibodies specific to CD40L. A number of samples were dripped with a pipette into the well so that the CD40L present in the sample would bind to the antibody. Washing was done to remove unbound substances, then polyclonal antibodies labeled with specific enzymes for CD40L are added to the well. The washing was performed again to remove the unbound enzyme-labeled antibody reagents.

The substrate was added and the color change occurs that measured after the addition of the reagent to stop the reaction. After that, the sample for serum sCD40L level examination. Serum samples were obtained from 5 ml of venous blood inserted into serum separator tube (SST). The sample was allowed to form the clot for 30 minutes before centrifugation at 1000g for 15 minutes.

The serum transferred into an aliquot tube and stored at a temperature of -20°C until examination. Serum samples were diluted 5 times by adding 50 μl samples with 200 μl diluent calibrator RD5P. Followed by preparing the reagent which the first left for some time at room temperature before use. The inspection procedure for sCD40L levels was expanded when all reagents, standards, and samples have been prepared. The microplate strip to be used as released from the intact microplate. The microplate was placed in a horizontal orbital shaker with a speed of 500 ± 50 rpm and incubated for 2 hours at room temperature.

The standard curve was made by inserting the absorbent of each standard solution on the y-axis and the level on the x-axis, then connecting the dots into a straight line. Nonlinear data can use absorbent logs and logs. The levels can be read with standard curves based on each absorbent. SCD40L sample rate was the multiplied reading result level 5.

All data on ACS patient characteristics, distribution, and frequency of ACS are presented descriptively. Diagnostic value of examination of sCD40L as the marker of ACS in Dr. Soetomo Surabaya obtained from the calculation of the formula to calculate the sensitivity, specificity, positive predictive value, and negative predictive value, using 2x2 tables. Statistical analysis to
determine the relationship between sCD40L and ACS incidence by using Pearson correlation test.11

Results

The lowest SCD40L test result of patients with chest pain was 0.07 ng/ml obtained in Stable Angina patients while the highest level was 12.39 ng/ml obtained in patients with NSTEMI diagnosis. The mean sCD40L level of chest pain patients was 4.23 ± 3.49 ng/ml. ACS patients had mean sCD40L levels of 5.45 ± 3.70 ng/ml, while non-ACS patients had mean sCD40L levels of 1.97 ± 1.33 ng/ml. The mean rate of patients with the diagnosis of ACS was significantly higher than the mean rate of patients with non-ACS diagnosis (p <0.05), whereas the mean sCD40L concentration in ACS subgroup did not find the significant difference (Table 1).

The SCD40L levels of ACS patients were 16 (61.53%) above the cut off (> 2.99 ng/ml) while 10 (38.47%) were obtained to be lower than cut off (<2.99 ng/ml). Most non ACS patients, 10 (71.42%) had sCD40L levels below cut off (<2.99 ng/ml), while the remaining 4 (0.28%) had sCD40L levels above cut off (> 2.99 ng/ml) (Table 2). The calculation of the diagnostic value of sCD40L based on 2x2 table showed 61.53% sensivitas, specificity 71.4%, predictive value 80% and 50% negative predictive value (Table 3).

Correlation analysis aims to determine the pattern and closeness of the relationship between two or more variables. Statistical analysis to determine the relationship between sCD40L level and ACS incidence using Spearman test because the sCD40L content data is not normally distributed. Result of statistical analysis got coefficient correlation rho (ρ) = 0.52 with value p = 0.001. This means that the sCD40L level was moderately correlated with the ACS incidence and this correlation was significant. The positive correlation coefficient indicates that the correlation was unidirectional, i.e., higher sCD40L levels will increase the incidence of ACS.

<table>
<thead>
<tr>
<th>Groups</th>
<th>sCD40L Level (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-ACS</td>
<td>1.97 ± 1.33*</td>
</tr>
<tr>
<td>ACS</td>
<td>5.45 ± 3.70*</td>
</tr>
<tr>
<td>UA</td>
<td>6.02 ± 3.31</td>
</tr>
<tr>
<td>NSTEMI</td>
<td>5.60 ± 4.52</td>
</tr>
<tr>
<td>STEMI</td>
<td>5.26 ± 3.55</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Groups</th>
<th>ACS</th>
<th>Non-ACS</th>
</tr>
</thead>
<tbody>
<tr>
<td>sCD40L(ng/ml)</td>
<td>≥2.99</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>&lt;2.99</td>
<td>10</td>
</tr>
</tbody>
</table>
Table 3. The diagnostic value of sCD40L for diagnosis of ACS

<table>
<thead>
<tr>
<th>Diagnostic value</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnostic sensitivity</td>
<td>61.53%</td>
</tr>
<tr>
<td>Diagnostic specificity</td>
<td>71.42%</td>
</tr>
<tr>
<td>Predictive value is positive</td>
<td>80%</td>
</tr>
<tr>
<td>Predictive value is negative</td>
<td>50%</td>
</tr>
<tr>
<td>Positive ratio is positive</td>
<td>2.15</td>
</tr>
<tr>
<td>Positive ratio is negative</td>
<td>0.54</td>
</tr>
</tbody>
</table>

**Discussion**

The results of mean random blood glucose levels in ACS patients were higher than non-ACS patients. This is in accordance with previous studies that mention that acute hyperglycemia occurs in about 50% of patients with ACS. Cardiovascular stress will trigger the release of catecholamine, cortisol, and glucagon hormones resulting in increased glucose and free fatty acids increased hepatic gluconeogenesis and decreased peripheral glucose uptake. Acute hyperglycemia will worsen the prognosis and improve the mortality of ACS patients with or without previous diabetes history.

There was no difference between total cholesterol, triglyceride, and LDL cholesterol in ACS and non-ACS patients. The mean rate of HDL cholesterol in ACS patients was lower compared with non-ACS patients. This is in accordance with studies suggesting that elevated levels of HDL cholesterol are a protective factor, while low HDL cholesterol levels increase the risk of atherogenesis.

SCD40L examination results in patients with chest pain have the diagnosis of ACS was significantly higher than that of chest pain patients with a non-ACS diagnosis. It was in accordance with previous studies suggesting that elevated sCD40L may be used as a marker for thrombotic inflammatory activity in ACS patients. However, elevated sCD40L levels cannot be used to distinguish the ST elevation of myocardial infarction (STEMI), Non-ST elevation myocardial infarction (NSTEMI), or Unstable Angina (UA).

Platelet activation is an important key to the development of ACS. Subendothelial collagen exposure to platelets causes the activation and secretion of several thrombotic and proinflammatory molecules during the acute phase of myocardial infarction. sCD40L is one of the proinflammatory molecules secreted by active platelets and involved in the process of plaque destabilization and thrombus formation.

Increased sCD40L levels were also one of the causes of ACS, which allows continuous cycles in ACS patients. The occurrence of elevated sCD40L levels is due to plaque rupture. The results of the use of sCD40L levels were lower than previous studies. Previous studies have suggested that the use of sCD40L for diagnosis of ACS in patients with chest pain has a sensitivity of 90% and a specificity of 86%. The reason for the difference between this study and the previous was the number of samples used.

Risk factors that may affect the occurrence of ACS were smoking history. Smokers tend to have higher levels of sCD40L compared to nonsmokers. If the patient has a history of previous diseases such as diabetes, hypertension, and dyslipidemia may affect the patient’s sCD40L level. Patients with a history of hypertension tend to have the CD40/CD40L system become more reactive so that sCD40L levels become higher. SCD40L levels will also increase in dyslipidemia patients. Other conditions of increased levels of sCD40L were autoimmune diseases, multiple sclerosis, inflammatory bowel disease, and stroke.

 Several ACS patients in this study had a history of using drugs such as Aspirin and Simvastatin. Studies show that there is no association between aspirin use and serum sCD40L levels. Aspirin is often used in patients
with angina and may inhibit some platelet function, but inhibition of sCD40L release requires a stronger platelet inhibitor, such as GP IIb/IIIa inhibitors. The use of drugs for the treatment of dyslipidemia and hypertension such as Simvastatin, Losartan, or a combination of both for 2 months can lower sCD40L.

The result of correlation of sCD40L with ACS incidence has the moderate correlation, and the higher the sCD40L cause the higher incidence of ACS. Studies suggest that elevated levels of sCD40L are an independent risk factor for the occurrence of death and recurrent myocardial infarction in ACS patients. Other studies suggest that elevated sCD40L levels will increase the risk of follow-up cardiovascular events, such as Acute myocardial infarction (AMI), sudden death, and recurrent angina in CHD patients.

**Ethical Clearance:** This research process involves participants in the survey using a questionnaire that was accordant with the ethical research principle based on the regulation of research ethic committee. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, nonmaleficence, and justice.

**Conflict of Interest:** The author reports no conflict of interest of this work.

**Source of Funding:** This study is done with individual funding.

**Conclusion:** Based on the results of analysis and discussion it can be concluded that sCD40L levels in patients with chest pain have diagnosed of ACS that significantly higher than patients with the non-ACS diagnosis. The use of sCD40L level for diagnosis of ACS in patients with chest pain was 61.53% sensitivity and 71.4% specificity. The correlation results obtained positive moderate correlation between sCD40L level and the incidence of ACS in patients with chest pain.

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The Potential Role of Radiology in Diagnosis of Traumatic Versus Non-Traumatic Cerebral Hemorrhages for Medicolegal Application

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Abstract

Background: Radiography is traditionally used as a complement tool for forensic examination. CT applications became widely used. Traumatic cerebral hemorrhage occurs in up to 15% of patients following head injury and non-traumatic cerebral hemorrhage accounts for up to 44% of all strokes. These two disorders are therefore very common. So there is a need for accurate diagnosis of cerebral hemorrhage form medicolegal aspect. This study aims to differentiate between traumatic and non-traumatic cerebral hemorrhage by using CT.

Methods: This prospective study, was conducted on 100 patients with intracranial hemorrhage and divided in two equal groups (traumatic and non-traumatic cerebral hemorrhage) both groups were examined by CT head and the results were analyzed.

Results: There was statistically significant difference between traumatic and non-traumatic cerebral hemorrhage groups in CT finding as regard type of hemorrhage with epidural, subarachnoid and intraventricular with P value (0.001, 0.005 and 0.001) respectively. Also according to site of hemorrhage in frontal lobe and subcortical with P value (0.001) equally.

Conclusion: we conclude that equation

\[ f = -0.976 \text{ (constant)} + 2.873 \text{ (Epidural)} + 2.017 \text{ (Subarachnoid)} - 0.416 \text{ (Intraventricular)} + 0.984 \text{ (Frontal)} - 3.047 \text{ (Subcortical)} \]

If the function \( f = + \) values, it will be traumatic hemorrhage and if the function \( f = - \) values it will be pathological hemorrhage.

Keywords: CT, traumatic, non-traumatic, cerebral hemorrhage, forensic

Introduction

Radiography is traditionally used as a complement tool for forensic examination, serving primarily to document metallic bullet fragments, foreign bodies, fractures, and injury patterns¹. CT applications became widely used and had been introduced as a routine investigation in forensic medicine².

The frequency of traumatic brain injuries is high among young children and elder age groups due to road traffic accidents, work or sport accidents, firearms, and falls from height³. On the other hand, non-traumatic cerebral hemorrhage caused by hypertension (36%), aneurysm (36%), arteriovenous malformation (11%) and other causes (17%)⁴.

There is a need for accurate diagnosis of different types of cerebral hemorrhage including epidural, subdural, subarachnoid and intracerebral hemorrhage with the increasing awareness of its high prevalence⁵. Imaging methods may be capable of detecting a number of the pathoanatomical and pathophysiological consequences of focal and diffuse traumatic brain
The location of the hemorrhage is very important in the diagnosis of the nature of the hemorrhage. Subdural hemorrhage and epidural hemorrhage are mainly traumatic, while a subarachnoid hemorrhage and intracerebral hemorrhage when not accompanied with bruises and lacerations of the brain it can be non-traumatic.

Radiological imaging such as CT offering a non-invasive approach and unlimited storage and can provide as a second opinion in the court.

Material and Method

This study is a prospective, case control study conducted on 100 patients with intracranial hemorrhage selected from cases presented to the Neurosurgery department, Faculty of medicine, Cairo University, from February 2017 to January 2018.

Intracranial hemorrhage may be non-traumatic or traumatic in nature, so the patients were divided into two groups according to history of trauma as following:

♦ **Group 1 (Traumatic):** 50 patients with history of trauma.

♦ **Group 2 (Non-traumatic):** 50 patients without history of trauma.

Patients’ Inclusion Criteria:
1. Both gender was included.
2. Different age groups ( > 18, 18-54, < 55).
3. Craniocerebral hemorrhage as a primary cause of neurological deficit regardless of the etiology.

Patients’ Exclusion Criteria:
1- Cases due to thrombotic embolization.
2- Cases due to intracerebral infection.
3- Cases due to congenital anomalies (Except vascular anomalies).
4- Cases due to neoplastic lesions unless the incidence is acute due to associated hemorrhage.

Cases were analyzed with respect to:
- patients age and sex
- Presence or absence of history of head trauma to classify cerebral hemorrhage to traumatic and non-traumatic
- In traumatic group: the cause of trauma as road traffic accident (RTA), assault, fall from height or others.
- Data from application of cranial CT on Toshiba Alexon 16-slice multidetector CT scanners to detect:
  - Cause of hemorrhage in pathological group aneurysm, hypertension (HTN) or arterovenous malformation (AVM).
  - Type of hemorrhage and combined types of hemorrhages.
  - Site of hemorrhage and combined sites of hemorrhages.

Statistical analysis:
- Data were coded and entered using the statistical package SPSS version 25. The comparisons between quantitative variables were done using the non-parametric Mann-Whitney test. And for comparing categorical data, Chi square (χ²) test was performed. Exact test was used instead when the expected frequency is less than 5. P-values less than 0.05 were considered as statistically significant.

Discriminant analysis was done to discriminate between pathological and traumatic. Stepwise statistics revealed the significant predictors which were used to determine the discriminate function. Then group centroids (group means) were calculated, they represent the determinant points for discrimination between traumatic and pathological. Classification of the percentage of accurately classified cases according to the discriminate function was performed.

Finding

This study was conducted on 100 patients with intracranial hemorrhage and divided into two equal groups according to history of trauma (traumatic group and non-traumatic group).

Results showed that the mean age was (33.78) years old in (Traumatic) and (49.30) years old in (non-traumatic).
Regarding gender the majority of the cases are males which represent (68%) and female cases represent (32%).

Regarding types of trauma in traumatic group the most common cause is RTA (42%) then followed by assault (34%) then followed by fall from height (20%).

According to pathological causes in non-traumatic group, the most common cause is aneurysm (54%) then followed by HTN (38%).

Table (1): Show comparison between Traumatic group and Non-traumatic group as regard type of hemorrhage according to CT finding. It shows statistical significant difference with epidural, subarachnoid and intraventricular with P value (0.001, 0.005 and 0.001) respectively.

<table>
<thead>
<tr>
<th>Type of hemorrhage on CT</th>
<th>Group</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Traumatic</td>
<td>Non-traumatic</td>
<td>P value</td>
</tr>
<tr>
<td></td>
<td>Count</td>
<td>%</td>
<td>Count</td>
<td>%</td>
</tr>
<tr>
<td>Epidural</td>
<td>yes</td>
<td>24</td>
<td>48.0%</td>
<td>0</td>
</tr>
</tbody>
</table>
|                          | no    | 26               | 52.0%            | 50     | 100.0%
| Subdural                 | yes   | 8                | 16.0%            | 9      | 18.0%
|                          | no    | 42               | 84.0%            | 41     | 82.0%
| Subarachnoid             | yes   | 20               | 40.0%            | 34     | 68.0%
|                          | no    | 30               | 60.0%            | 16     | 32.0%
| Intracerebral or Intraparenchymal | yes | 9              | 18.0%            | 12     | 24.0%
|                          | no    | 41               | 82.0%            | 38     | 76.0%
| Intraventricular         | yes   | 0                | 0.0%             | 11     | 22.0%
|                          | no    | 50               | 100.0%           | 39     | 78.0%

Table (2): Show comparison between Traumatic group and Non-traumatic group as regard site of hemorrhage according to CT finding. It shows statistical significant difference in frontal lobe and subcortical with P value (0.001) equally.

Table (2): Comparison between two groups as regard sites of hemorrhage:
The patients with single site of hemorrhage were higher in Traumatic group where the patient with combined site of hemorrhage were higher in Non-traumatic group with statistical significant difference.

**Table (3,4):** Show discriminant function analysis to discriminate between non-traumatic and traumatic cerebral hemorrhage using CT findings, we collect significant parameter in analysis in table 1 (types of hemorrhage) which is epidural, subarachnoid and intraventricular hemorrhage and significant parameter in analysis in table 2 (sites of hemorrhage) which in frontal and subcortical, then we done discriminant function analysis model. (Table 3)

**Table (3): Discriminant Function Coefficients:**

<table>
<thead>
<tr>
<th>Site of hemorrhage</th>
<th>Group</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Traumatic</td>
<td>Non-traumatic</td>
</tr>
<tr>
<td></td>
<td>Count</td>
<td>%</td>
</tr>
<tr>
<td>Temporal</td>
<td>yes</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>33</td>
</tr>
<tr>
<td>Frontal</td>
<td>yes</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>36</td>
</tr>
<tr>
<td>Pareital</td>
<td>yes</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>24</td>
</tr>
<tr>
<td>Occipital</td>
<td>yes</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>43</td>
</tr>
<tr>
<td>Subcortical</td>
<td>yes</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>50</td>
</tr>
</tbody>
</table>

Function (f) = - 0.976 (constant) + 2.873 (Epidural) + 2.017 (Subarachnoid) - 0.416 (Intraventricular) + 0.984 (Frontal) -3.047 (Subcortical)

**Table (4): Functions at Group Centroids:**

<table>
<thead>
<tr>
<th>Group</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-traumatic</td>
<td>-1.485-</td>
</tr>
<tr>
<td>Traumatic</td>
<td>1.485</td>
</tr>
</tbody>
</table>

According the result of previous equation to functions at group centroids (table 4) which showing that non-traumatic (-1.485) and traumatic (1.485). So we conclude that if the function (f) = + values (> 0) it was traumatic hemorrhage and if the function (f) = - values (< 0) it was non-traumatic hemorrhage.

**Table (5):** Shows classification to show accuracy of predicted group membership by discriminant function analysis, we found that (90%) of cases were truly classified as 44 patients of non-traumatic cases which represent (88%) were truly classified as non-traumatic and 46 patients of traumatic cases which represent (92%) were truly classified as traumatic with accuracy of that model is 90%.
Table (5): Classification to show accuracy of predicted group membership by discriminant function analysis:

<table>
<thead>
<tr>
<th>Group</th>
<th>Original Count</th>
<th>Predicted Group Membership</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Traumatic</td>
<td></td>
</tr>
<tr>
<td>Non-traumatic</td>
<td>44</td>
<td>6</td>
<td>50</td>
</tr>
<tr>
<td>Traumatic</td>
<td>4</td>
<td>46</td>
<td>50</td>
</tr>
<tr>
<td>%</td>
<td>Non-traumatic</td>
<td>88.0</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>Traumatic</td>
<td>8.0</td>
<td>92.0</td>
</tr>
</tbody>
</table>

**Discussion**

Traumatic cerebral hemorrhage and non-traumatic cerebral hemorrhage are therefore very common\(^2\). So the accurate diagnosis of cerebral hemorrhage is important from medicolegal aspect.

The study showed that the mean age was (33.78) years old in Traumatic group and the mean age was (49.30) years old in Non-traumatic group.

These results were in agreement with those of Zhou\(^3\) who found that the incidence of acute spontaneous intracranial hemorrhage tended to increase with increasing age, the incidence of traumatic brain injury tended to decrease with decreasing age.

Regarding sex, it was found that male patients were higher which represent (68%) and female cases represent (32%).

These results were in agreement with those of Pruitt\(^4\) who found that (61.1%) of patients were males.

Also it was found in the present study that male patient are higher in Traumatic group and female patient are higher in Non-traumatic group. This result agrees with those of Al-Yessary\(^5\) who found males were more frequent among traumatic group, while females were the more frequent gender in spontaneous group and it was explained according to van Beijnum\(^6\) that the traumatic intracranial hemorrhage had mostly occurred in males because they usually expose to violence and trauma more than females. Spontaneous intracranial hemorrhage had mostly occurred in females, and that may be due to their higher reaction to emotional stress than that of males.

Regarding the type of trauma in traumatic group in the present study, it was found that the most common cause of traumatic intracranial hemorrhage is road traffic.

This disagrees with the study conducted by Pruitt\(^4\) who found the most common cause of injury was fall which represent (69.6%).

It was noticed that different of most common cause of head injury from country to another. It was explained by Odero\(^7\) as he mentioned that increasing rate head injury in developing nations due to increasing incidence of road traffic accidents.

Regarding the pathological causes in Non-traumatic group, it was found the most common cause of non-traumatic intracranial hemorrhage was aneurysm (54%) then followed by HTN (38%).

According to Van\(^8\) the majority causes of nontraumatic intracranial hemorrhage was hypertension (36 %), aneurysm (36 %) with equally percent.

Regarding type of hemorrhage, it was found that patients with epidural hemorrhage was more common in Traumatic group while patients with subarachnoid and intracerebral were more common in Non-traumatic group.

These results were in agreement with those of Suthar\(^9\) as they found in their study subarachnoid hemorrhage,
intracerebral hemorrhage and intraventricular hemorrhage were more common in spontaneous intracranial hemorrhage and epidural hemorrhage and subdural hemorrhage were more common in traumatic intracranial hemorrhage.

Regarding combined types of hemorrhage, it was found that patients without combined types of hemorrhage (one type) were (82%) in Traumatic group and (72%) in Non-traumatic group and patients with combined types of hemorrhage (18%) in Traumatic group and (28%) in Non-traumatic group.

These results were in agreement with those of Pruitt\textsuperscript{14} as they found that (74.3%) of traumatic patients had a single type of intracranial lesion versus (24%).

Regarding site of hemorrhage in the present study, it was found that Patients with hemorrhage in frontal lobe were (28%) in Traumatic group and (4%) in Non-traumatic group and patients with hemorrhage in subcortical (0%) in Traumatic group and (60%) in Non-traumatic group.

These results were in agreement with those of Suthar\textsuperscript{5} as they found in their study that the most common site of hemorrhage was basal ganglia (subcortical) (60%) in spontaneous intracranial hemorrhage.

Our results showed that most common site in traumatic intracranial hemorrhage was parietal, frontal, and temporal. These results were in agreement with those of Adekanmi\textsuperscript{19} who found that most of the bleeds were located in the parietal and frontal lobes, followed by temporal.

### Conclusion

From the present study, it was concluded that:

- Males were more commonly affected by cranial hemorrhages than females and higher in traumatic group but females were higher in non-traumatic group and the traumatic patients were younger than non-traumatic patient.
- The most common cause of traumatic intracranial hemorrhage is RTA followed by assault and the most common cause of non-traumatic intracranial hemorrhage is aneurysm then followed by HTN
- The most common type of hemorrhage in traumatic group was epidural while in non-traumatic group was subarachnoid hemorrhage.
- The most common site of hemorrhage in traumatic group was in parietal while in non-traumatic was in subcortical.
- The patients with single site of hemorrhage were higher in traumatic group while patients with combined site of hemorrhage were higher in non-traumatic group.
- We conclude the most important and significant finding in CT brain and we done discriminant function analysis model to discriminate between traumatic and non-traumatic cerebral hemorrhage and we found that equation

\[
\text{Function (f) = - 0.976 (constant) + 2.873(Epidural) + 2.017 (Subarachnoid) - 0.416 (Intraventricular) + 0.984 (Frontal) -3.047 (Subcortical)}
\]

According to function at group centroids we found non-traumatic was (-1.485) and traumatic was (1.485), so if we have the function (f) = + values (> 0) most probably it will be traumatic hemorrhage and if the function (f) = - values (< 0) most probably it will be non-traumatic hemorrhage as the accuracy of that model is 90%.

**Conflict of Interests:** The authors declared that they have no competing interests

**Ethical approval:** The study work was conducted after the approval of Ethical Committee, Faculty of medicine, Cairo University

**Source of Funding:** Self

### References


Comparison of Saffron versus Fluoxetine in Treatment of Women with Premenstrual Syndrome: A Randomized Clinical Trial Study

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Abstract

Background: Premenstrual syndrome (PMS) is a set of physical and psychological symptoms such as mood disability, breast tenderness, food craving, fatigue, and depression. Fluoxetine and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) usually are being administered for these patients. This study aimed to evaluate the effect of saffron plant in terms of its anti-inflammatory and anti-depressant effects compared to fluoxetine.

Materials and Method: This study was a three-blind clinical trial that was carried out on working women and their relatives. Firstly, 164 patients with the premenstrual syndrome were selected, and they were randomly divided into two 82-person groups, including those who received fluoxetine and those who received saffron. Next, both groups were treated for two months. The data were collected in two stages through a self-designed questionnaire (on day 5 of menstrual cycle) and validated questionnaires of PRISM and Beck at the end of the period.

Results: It was indicated that similar to fluoxetine, the use of saffron in PMS reduced the symptoms such as abdominal bloating, depression, and mood swing, but the latter could better relieve the breast and abdominal pain than fluoxetine.

Conclusion: It was concluded that the use of medicinal herbs such as saffron could be effective in reducing the symptoms and they might cause fewer side effects than chemical drugs.

Keywords: Saffron, fluoxetine, premenstrual syndrome.

Introduction

PMS is a type of mild to moderate neuropsychological disorder. Although its mild type occurs in 90% of cases, it is argued that its severe type occurs only in 5% of cases. PMS is a collection of physical, psychological and emotional symptoms associated with the menstrual
cycle in women. The syndrome begins 7 to 10 days before the start of menstruation, and it must be recorded in 2 consecutive cycles. It is associated with a set of physical and psychological symptoms such as mood swings, sensitization of breast, food craving, fatigue, irritability and depression. It is estimated that three swings, sensitization of breast, food craving, fatigue, physical and psychological symptoms such as mood in 2 consecutive cycles. It is associated with a set of before the start of menstruation, and it must be recorded in their late 30s and 40s.

Serotonin Reuptake Inhibitors (SSRIs), including Fluoxetine (Prozac, Sarafem), Paroxetine (Paxil, Pexeva), Sertraline (Zoloft), etc usually are recommended for the treatment of PMS. These drugs have been effective in improving symptoms such as fatigue, food cravings, and sleep disorders, and they are among the first choices to treat severe types of PMS.

Moreover, the consumption of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), diuretics as well as lifestyle changes are suggested for these patients. Some herbal medicines can also be effective in treating this syndrome. Saffron plant is one of these herbal medicines that is characterized by some anti-inflammatory and anti-depressant effects and, thus, it can be useful in treating this syndrome. Since the symptoms of the syndrome disrupt everyday life, it is beneficial to identify the ways of treatment, straightforward, harmless, and accessible treatments. Saffron has known anti-nociceptive, relaxing and anti-inflammatory effects as well as anti-depressant effect on humans and animals. It was attempted to examine the impact of this Iranian traditional and valuable herb in comparison with fluoxetine as a relatively well-known drug in the treatment of psychiatric disorders and even PMS. The purpose of this study was to reduce the complications of chemical medicines and medicinal herbs.

**Materials and Method**

This study was an interventional three-blind trial). This means that the patient, physician, and statistical analyst did not know the contents of the capsules (that was carried out on women employed in Deputy of Health of Sabzevar University of Medical Sciences, Occupational Medicine Center as well as their relatives. Regarding this, 164 employees who had approved a PMS based on two previous menstrual cycles and Hamilton Test were randomly selected, and written consents were obtained from them. Treatment included 20 mg capsules of fluoxetine in the control group and 30 mg capsules of saffron in the intervention group. The capsules were identical in appearance. The treatment allocation was randomly divided into two 82-person groups, and the drug packages were provided to patients by a person who did not know the contents of the drug packages. The two groups were treated in parallel. The duration of the treatment was two months, and the forenamed drugs should be taken daily from day 14 to day 28 of the menstrual cycle (within 14 days of the follicular phase). During the last 5 days of each cycle, the researchers attempted to record the concerned data using a self-designed checklist. During the treatment period, it was attempted to make sure that each patient made use of the prescribed drugs and that no associated drugs were used accordingly and no displacement occurred between the two groups. Furthermore, patients were reminded that they could contact the researchers if they had any new drug-related problems. It was also stipulated that if severe symptoms of depression occurred, which coerced the patients to use other drugs accordingly, the patients would be excluded from the study. Besides, if patients in the intervention group were obliged to use fluoxetine for some reason, these matters would be considered when analyzing the control group. At the end of each treatment period, it was attempted to make use of Beck Anxiety Inventory in order to determine the severity of anxiety and depression among patients. This questionnaire was a 21-point scale in which the patient chose one of four options in each item, and it indicated the severity of his/her anxiety. Four options of each question were scored in a four-part range from 0 to 3. Each test item described one of the common symptoms of anxiety (mental, physical and panic symptoms). The maximum score of this questionnaire was 63, and it was indicated that scores located between 0 and 7 were normal, scores located between 8 and 15 were mild, scores located between 16 and 25 were medium, and scores located between 26 and 63 were severe thereof. In addition, Visual Analogue Scales (VAS) was used to assess the severity of abdominal and breast pain (scores less than 3 showed mild abdominal pain, scores located between 4 and 7 indicated medium abdominal pain and scores located between 8 and 10 showed severe abdominal pain). In examining the amount of abdominal bloating, the patients were asked to measure their abdominal circumference (actually, patients were trained accordingly) (0-1 cm = unchanged, 1-2 cm = mild, 2-3 cm = medium and 3-4 cm = severe). Finally, the data were analyzed through Chi-square Test using...
Results

In this study, 164 people participated (each group was 82 individuals). The mean age of the patients was 35.5 ± 13 years (Range: 26-45). After comparing the two groups, it was shown that the age distribution was similar in both groups (the age distributions in the intervention and control groups were 36.5 ± 10 and 34 ± 5.30 years old, respectively). It should be noted that all patients were married.

Having examined the abdominal pain using the Visual Analogue Scales, it was found that there was no significant difference in the improvement of abdominal pain in the two groups (P>0.05). Having compared the severe to moderate abdominal pain, there was a significant difference between the two groups at the end of the first month (p = 0.01) and the second month (p = 0.03) (Table 1).

* Given the anxiety level, it was declared that there was no significant difference in the reduction of anxiety level between the two groups (P>0.05). Having compared the severe to moderate anxiety, it was indicated that despite significant reduction in anxiety level after taking drugs, notably saffron, there was not a significant difference between the two groups at the end of the first month (p = 0.83) and the second month (p = 0.09) (Table 2).

Comparing the breast pain in saffron group with fluoxetine, it was indicated that there was a significant difference between the two groups at the end of the first month (p = 0.0001), but there was not a significant difference between the two groups at the end of the second month (p = 0.014) (Table 3).

It was shown that there was no significant difference between the two groups according to depression(P>0/05) (Table 4).

In the study of mild, medium and severe abdominal bloating, there was no significant difference between the two groups at the end of the first and second months (P>0/05) (Table 5).

The side effects of saffron were not observed during the study.

Registration Number in the Clinical Practice Center of the Ministry of Health and Medical Education: IRCT 2017061233202N2

Discussion

PMS is a collection of physical, psychological and emotional symptoms associated with menstrual cycle in women. The syndrome begins 7 to 10 days before the start of menstruation, and it must be recorded in 2 consecutive cycles. Although its mild type occurs in 90% of cases, it is argued that its severe type occurs only in 5% of cases. It is associated with a set of physical and psychological symptoms such as mood swings, sensitization of breast, food craving, fatigue, irritability and depression 1. It is estimated that three women (out of 4 women) will experience this syndrome in their menstrual cycle. Some women experience this syndrome at the age of 20, but others suffer from this problem in their late 30s and 40s 2, 3. Many medicines are proposed to treat this syndrome, and some herbal medicines have been effective in treating this syndrome. Saffron plant is one of these herbal medicines that is characterized by some anti-inflammatory and anti-depressant effects and, thus, it can be effective in treating this syndrome. Since the symptoms of the syndrome disrupt everyday life, it is beneficial to identify the ways of treatment, especially simple, harmless and accessible treatments 1-7. Considering the known anti-nociceptive, relaxing and anti-inflammatory effects of saffron extract as well as its anti-depressant effect on humans and animals 10, it was attempted to examine the effect of this Iranian traditional and valuable herb in comparison with fluoxetine as a relatively well-known drug in the treatment of psychiatric disorders and even PMS.

This study was an interventional three-blind trial that was carried out on 164 women as well as their relatives. The most common age group afflicted with the PMS was those patients aged 16 to 45 years old. Compared to fluoxetine, it was indicated that if patients consumed saffron, their symptoms of abdominal and breast pain were alleviated better and more efficiently. However, there was no significant difference between the two groups in terms of reduction in anxiety level.

Mokhber et al. (2004) reported that the improvement in symptoms of depression, emotional dysfunction, appetite changes, lack of self-control, decreased interest in doing activities, concentration disorders, and sleep changes were significantly higher in fluoxetine-treated patients 13. They also evaluated the improvement of physical symptoms and tiredness and asserted that there
was no significant difference between placebo and fluoxetine.  

In our study, the intake of fluoxetine and saffron alleviated the depression and anxiety symptoms, but there was no significant difference between them. Moreover, the present study indicated that, compared to fluoxetine, saffron could better alleviate the symptoms of abdominal and breast pain.

Besides, Agha-Hosseini et al. found that, compared to placebo, saffron significantly improved the symptoms of PMS (up to 50%). Similarly, the present study showed that saffron could significantly alleviate the symptoms of PMS (i.e., depression, abdominal bloating, abdominal pain, breast pain, and anxiety).

Kashani et al. (2017) reported that saffron acted similar to fluoxetine in alleviating depression. They also found that saffron is a safe alternative medication for improving depressive symptoms of postpartum depression. Similarly, the results of the present study confirmed this anti-depressant effect of saffron.

One of the strengths of this study was to investigate the effects of saffron on patients who were all married. Inevitably, this homogenization reduced the impact of interfering factors.

Accordingly, it can be argued that the administration of saffron in the follicular phase of menstruation has a better effect on alleviating the severity of symptoms of the PMS than fluoxetine.

Considering the fact that pharmaceutical drugs and chemicals poisoning are common in Iran, considering traditional plant may be a better option in the PMS.

**Conclusion**

It was concluded that the use of medicinal herbs such as saffron could be effective in reducing the symptoms and they might cause fewer side effects than chemical drugs.

**Conflict of Interests:** None.

**Funding:** The research project of this manuscript was supported financially by Sabzevar University of Medical Sciences

**Ethical Clearance:** This study was approved by the ethics committee of Sabzevar university of medical sciences.

### Table 1. Frequency of patients with moderate to severe abdominal pain.

<table>
<thead>
<tr>
<th>Abdominal pain (medium-severe)</th>
<th>The end of first month</th>
<th>The end of second month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoxetine</td>
<td>64 patients</td>
<td>9 patients</td>
</tr>
<tr>
<td>Saffron</td>
<td>47 patients</td>
<td>0 patient</td>
</tr>
</tbody>
</table>

### Table 2. Frequency of patients with moderate to severe mood swing.

<table>
<thead>
<tr>
<th>Mood swing</th>
<th>The end of first month</th>
<th>The end of second month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoxetine</td>
<td>74 patients</td>
<td>44 patients</td>
</tr>
<tr>
<td>Saffron</td>
<td>71 patients</td>
<td>0 patient</td>
</tr>
</tbody>
</table>

### Table 3: The comparison of breast pain measured by Visual Analogue Scales (VAS) in two groups.

<table>
<thead>
<tr>
<th>Breast pain</th>
<th>The end of first month</th>
<th>The end of second month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoxetine</td>
<td>73 patients</td>
<td>27 patients</td>
</tr>
<tr>
<td>Saffron</td>
<td>32 patients</td>
<td>0 patient</td>
</tr>
</tbody>
</table>
### Table 4. Frequency of patients with depression (moderate to severe) diagnosed by the physician.

<table>
<thead>
<tr>
<th>Depression</th>
<th>The end of first month</th>
<th>The end of second month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoxetine</td>
<td>28 patients</td>
<td>0 patients</td>
</tr>
<tr>
<td>Saffron</td>
<td>28 patients</td>
<td>0 patient</td>
</tr>
</tbody>
</table>

### Table 5. Frequency of patients with moderate to severe abdominal bloating.

<table>
<thead>
<tr>
<th>Abdominal bloating</th>
<th>The end of first month</th>
<th>The end of second month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoxetine</td>
<td>34 patients</td>
<td>4 patients</td>
</tr>
<tr>
<td>Saffron</td>
<td>27 patients</td>
<td>1 patient</td>
</tr>
</tbody>
</table>

### References


Comparison of the Effects of Passiflora Incarnata and Piroxicam in opioids withdrawal-Induced Myalgia and Anxiety: A randomized Clinical Trial

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Abstract

Background: Non-steroidal analgesics are the most commonly used analgesics for pain relief in opioids induced withdrawal. One of the herbal remedies that reduce muscle pain, anxiety, and fatigue is Passiflora Incarnata (P. incarnata). In this study, we compared Piroxicam and P. incarnata in withdrawal-induced myalgia and anxiety.

Materials and Method: This was a clinical trial carried out on 43 candidate patients for maintenance treatment with methadone. The individuals were randomly divided into two groups. The control group was prescribed piroxicam capsule (10 mg every 12 hours), while the intervention group received drops of P. incarnata (10-15 drops three times a day), until symptoms of withdrawal were resolved (7 to 10 days). In the first, 3rd, sixth, and ninth days of treatment, the patient’s vital signs, myalgia (visual acuity), and anxiety (Beck test) were recorded and analyzed.

Results: The results of this study revealed that the flower drops resulted in a reduction in myalgia (mean score of 5.4± 0.79 on the first day decreased to 1.3 ±0.5 on the ninth day), but there was no significant difference in comparison with Piroxicam (p=0.705). Further, P. incarnata significantly reduced the anxiety caused by withdrawal (p =0.001).

Conclusion: P. incarnata drop can be used to reduce the pain and anxiety caused by the drug’s withdrawal.

Keywords: Passiflora Incarnata, Myalgia, Anxiety, Addiction withdrawal.

Introduction

Today, drug addiction and opioids poisoning are social, health, and political problem involving most countries of the world, especially the developing countries. Opioids use has so many direct or indirect adverse on human health. Unfortunately, drug addiction and poisoning in adolescence and youngsters also are increasing. Methadone is a proper replacement for withdrawal situation. Withdrawal is also accompanied by important consequences such as anxiety, stress, nervous seizures, and severe myalgia. Tolerating myalgia is hard and unbearable for an addicted person.

Non-steroids are one of the most commonly used analgesics to reduce the pain caused by withdrawal. While based on Iranian traditional medicine, one of the herbal remedies that reduce myalgia, anxiety, and...
grouch is *Passiflora incarnata*\(^\text{15}\).

*Passiflora incarnata* L. (Passifloraceae) is used in traditional herbal medicines, which has shown different therapeutic properties \(^\text{16, 17}\). Interestingly, in India, it is used to treat morphine’s dependence \(^\text{18}\), while in Africa, it is used for its sedative and analgesic properties \(^\text{19, 20}\). The *P. incarnata* is not narcotic or addictive, so it is used in tea, pills, and drops for treatment of sleep disorders, restlessness, irritability, and anxiety \(^\text{15, 21, 22}\). In recent years, many studies have been conducted on the effective substances of this plant as anti-anxiety and toxicity properties \(^\text{23-25}\), and as a benzodiazepine-receptor agonist \(^\text{24}\). Animal studies have also revealed that *P. incarnata* L. has been effective in controlling the pain caused by alcohol withdrawal \(^\text{20}\). Meanwhile, other studies have indicated that when the plant is used in the treatment, it reduces the need for opioids in addicts \(^\text{26}\).

Piroxicam is a potent inhibitor of the cyclooxygenase enzyme with some serious adverse effects \(^\text{27}\). Regarding the effects of analgesic drugs and very few studies on the effectiveness of *P. incarnata* plant in some common complications of drug addiction withdrawal, this study was conducted to compare the effects of Piroxicam and *P. incarnata* on myalgia and anxiety due to addiction withdrawal.

**Materials and Method**

This project was a single-blind clinical trial performed on clients of addiction treatment centers in Sabzevar city. Conscious informed consent form was obtained from all participants in the study. The project was also registered as IRCT20170404033202N6 at the Iranian Center for Clinical Record Registration. Forty-three patients who wanted to replace use of opium with methadone were included into the project with personal satisfaction. The subjects were divided into two groups using simple random method. Piroxicam (10 mg once every 12 hours) was prescribed to one group (\(n = 20\)), while the other group (\(n = 23\)) received *P. incarnata* drop (10-15 drops based on the weight three times a day) \(^\text{28, 29}\), which were consumed until the withdrawal symptoms resolved and methadone dosage stabilization (between 7 and 9 days).

The patients received methadone and analgesic every day, until resolution of withdrawal symptoms and methadone dose stabilization. Every three days (1st, 3rd, sixth and ninth), the patient’s vital signs were based on a self-designed checklist; the amount of muscle pain was completed based on the Visual analog scale (VAS) and anxiety based on the Beck test. If the visual score of pain was more than 9, then pain reliever would begin and be excluded from the study and another person would be replaced. The Beck Anxiety Questionnaire has a high validity rating \(^\text{20}\). The total score of this questionnaire lies within the range from 0 to 63 \(^\text{30}\). The pain was measured by the researcher using Visual Analog Scale (VAS), standardized for pain assessment.

Finally, the data were collected and analyzed by SPSS20 through independent T-tests, paired t-test, and repeated measurement.

**Results**

In the study of 43 individuals selected in this study, the mean age of the subjects was \(35.7 \pm 3.5\) years in the two groups, with a total of at least 28 years and a maximum of 45 years old, where most patients were 30 to 42 years old. The mean age in *P. incarnata* group was \(37 \pm 4\) years and \(34.5 \pm 3\) years in the Piroxicam group (Table 1).

All subjects were male, so in terms of the threshold of pain, there is no confounding. The frequency of drug use was between 1 and 3 times a day, with an average of \(2.4 \pm 0.2\) mg/dl in the intervention group, and in the control group \(2.3 \pm 0.2\) times a day. Most of the patients used opium three times a day. The mean amount of consumed drug (opium) was \(1.28 \pm 0.5\) in the intervention group, and \(1.21 \pm 0.5\) in the control group. The duration of opium addiction varied from 1 to 4 years, with an average of 2.68 years. The number of drug intake days (passiflora or piroxicam) was between 7 and 9 days until all the symptoms of pain were resolved. A total of 41% of people had 8 days of taking medication (pain reliever). The mean of Piroxicam and Passiflora use was \(8 \pm 0.79\) and \(7.96 \pm 0.76\), respectively (\(p = 0.85\)). The mean score of pain and anxiety before the intervention was not significantly different between the two groups (\(p > 0.05\)).

In the Piroxicam group, the mean score of the VAS on the first day was \(0.86 \pm 4.7\), and on the ninth day, it was \(0.51 \pm 0.1\), which showed a significant decrease (\(p < 0.001\)). In the *P. incarnata* group, pain intensity was \(0.79 \pm 5.4\) on the first day and decreased to \(0.5 \pm 1.3\) on the ninth day (\(p < 0.001\)).

Comparison of the two groups on the 9th day in the Piroxicam group revealed a mean pain score of 0.51 ± 1.4
and in passiflora group 0.5 ± 1.3 (p = 0.705). The mean anxiety score in the Piroxicam group on the first day was 0.5 ± 3.50 and on the ninth day in the same group, which was 0.5 ± 2.4 (p = 0.001). In the passiflora group, the average anxiety score on the first day of treatment was 0.5 ± 3.62, and on the ninth day of treatment, it was 0.34 ± 1.1 (p = 0.010). Comparing the effect of two drugs on anxiety, the results indicated that the mean anxiety score in the Piroxicam group was 0.5 ± 2.4 on the ninth day, and 0.34 ± 1.1 in the passiflora group (p = 0.001). Based on the results of repeated measurement analysis, pain and anxiety severity at different times in the \( P. \) \( \text{incarnata} \) group and piroxicam group were statistically significant (P <0.001). Further, the results of Bonferroni post hoc test showed that all the times in each group had a significant difference in two groups (P <0.05) (Table2).

**Table 1: Comparison of demographic variables and data related to addiction in two groups of Piroxicam and Passiflora incarnata**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Passiflora incarnata group</th>
<th>Piroxicam group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>4 ± 37</td>
<td>3 ± 5/34</td>
</tr>
<tr>
<td>Gender</td>
<td>Male 23</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Female 0</td>
<td>0</td>
</tr>
<tr>
<td>Duration of opium addiction (year)</td>
<td>2.81 ± 0.5</td>
<td>2.56 ± 0.5</td>
</tr>
<tr>
<td>Amount of opium use (g)</td>
<td>1.28 ± 0.5</td>
<td>1.21 ± 0.5</td>
</tr>
<tr>
<td>Drug use frequency per day</td>
<td>2.4 ± 0.2</td>
<td>2.3 ± 0.2</td>
</tr>
</tbody>
</table>

**Table 2: Comparison of severity of pain and anxiety in the designated days in two groups of passiflora and piroxicam**

<table>
<thead>
<tr>
<th>Time Group</th>
<th>Anxiety Score</th>
<th>Intensity of pain</th>
<th>repeated measurement test results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st day</td>
<td>3rd day</td>
<td>6th day</td>
</tr>
<tr>
<td></td>
<td>1st day</td>
<td>3rd day</td>
<td>6th day</td>
</tr>
<tr>
<td>Passiflora group (n=23)</td>
<td>3.6±0.5*</td>
<td>1.4±0.59*</td>
<td>1.13±0.34*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F=264.87 P&lt;0.001</td>
</tr>
<tr>
<td>Piroxicam group (n=20)</td>
<td>3.5±0.5*</td>
<td>3.2±0.52*</td>
<td>2.9±0.3*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F=36 P&lt;0.001</td>
</tr>
</tbody>
</table>

* Significant difference between each day and other times

**Discussion**

The results of this study suggested that drops of \( P. \) \( \text{incarnata} \) reduce the anxiety and myalgia caused by withdrawal. There was no statistical difference in the treatment of medication between the two treatments at the end of the study. To the best of our knowledge, this study is the first clinical trial on the effect of \( P. \) \( \text{incarnata} \) in the treatment of anxiety and myalgia caused by opium withdrawal. Compared to other similar studies that had examined the effect of passiflora on other diseases, there was a similarity between our study and those articles in terms of the sample size, duration of drug use as well as the results, which are in line with the findings of this study. Other studies showed the positive effects of Passiflora drops in reducing the anxiety similar to benzodiazepines; the greatest effects of \( P. \) \( \text{incarnata} \) was from day seven onward \(^{31,33}\). Some animal studies have also suggested the agonist effects on GABA-a in creating anti-anxiety effects \(^{23,24}\). Others also argue that \( P. \) \( \text{incarnata} \) extracts may be helpful in the control of withdrawal symptoms of alcohol consumption \(^{30}\). Some studies have suggested that opioid withdrawal leads to a significant reduction in the analgesic threshold and induction of withdrawal hyperalgesia \(^{35}\). Previous studies have reported the clinical role of active ingredients of this substance synergistically \(^{17}\). It is reasonable that the pain reliever and anti-anxiety properties of this plant can be attributed to the synergistic action of GABA.
A, GABA B and its effect on opioid receptor 20, 36. A considerable part of the scientific literature has provided pre-clinical evidence of the beneficial properties of P. incarnata in the treatment of addictive behaviors related to substances such as amphetamine, nicotine, cannabis, ethanol, and benzodiazepine 34,37,38.

**Conclusion**

The results suggested that P. incarnata drops as well as Piroxicam can reduce the severity and anxiety and myalgia caused by withdrawal.

**Conflict of Interests:** None.

**Funding:** The research project of this manuscript was supported financially by Sabzevar University of Medical Sciences.

**Ethical Clearance:** This study was approved by the ethics committee of Sabzevar university of medical sciences.

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Hematological Changes in Blood of Smokers of Cigarettes and Nargyile

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1University of Anbar-College of Science, 2Ministry of Education-Anbar

Abstract

The current study aimed at determining the effect of smoking cigarette and Narghile at some of bloody variables and Vitamin D in a sample of young smokers aged 20-35 in Ramadi. The study included complete blood count (C.B.C) tests including: Total White Blood Cell and Red Blood Cell Count, determination of the concentration of total hemoglobin and calculation of Platelet Count. Calculation of RBC Indicators: “mean corpuscular hemoglobin” (MCH), “Mean Corpuscular Hemoglobin Concentration” (“MCHC”) and Mean red blood Cell Volume (MCV). The study also evaluated the concentration of vitamin D.

Results showed that: A rise in the number of white blood cells and red blood cells, as well as a high concentration of hemoglobin and platelet count in smokers. An increase in the volume of red blood cells, mean hemoglobin concentration, and Mean Corpuscular Hemoglobin Concentration in smokers’ blood. Decreased concentration of vitamin D.

Key Words: Complete Blood Count, RBCs indices, Smokers, Cigarettes, Narghile

Introduction

Smoking is one of the leading causes of cancer throughout the body and is one of the causes of global confirmed deaths. Many studies have found that smoking is a major cause of many human diseases (1).

It is a global problem that causes 6.4 million deaths in 2015 and is expected to increase to 8.3 million by 2030 (2).

Tobacco contains organic substances such as alkaloids, nicotine, and other substances that have the same nicotine effect as arsenic, mercury, lead, cadmium, chromium, polonium and beryllium (3).

These substances negatively affect the physiological and biochemical functions of the body directly (4).

Cigarette smoking and narghile are one of the most important risk factors for heart disease and atherosclerosis, which accelerates the development of heart disease, which ends in death because of the Cigarette contained in the toxic substances whose cumulative effect appears over time (5).

Cigarette smoking is a significant proportion of myocardial infarction (MI). This is three times higher in non-smokers and the mechanism of effect is not fully known (6).

Despite the lack of knowledge of the mechanism in which smoking affects the rate of increase in the incidence of various heart diseases, but the containment of tobacco to thousands of toxic chemicals and harmful to health in general is the cause of the disease (7).

Smoking is a factor in causing neoplastic neoplasms and leads to infertility in men. Nicotine is also a substance that causes vasoconstriction, which leads to diminished blood vessels and reduced blood flow (8).

Smoking is a link between blood variables and cases of myocardial infarction (MI) as well as Coronary Heart Disease (CHD) by increasing the viscosity of the blood and associated with cases of narrowing of the blood vessels, which causes the breakdown of platelets and the release of enzymes into the bloodstream causing thrombosis (9).

Smoking causes osteoporosis, because it reduces the absorption of calcium from the small intestine as well as
increases the toxic substances of the bones and affects the metabolism of hormones and vitamins, including vitamin D.\(^{(10)}\).

The researchers note that there is a very strong association between smoking and cellular blood changes. This change leads to the stimulation of various heart diseases, including atherosclerosis and cardio-vascular disease\(^{(11)}\).

Vitamin D is known as the sun’s vitamin because the sun’s rays contain UV-B, which converts the 7-dehydroxy cholesterol into 25-dehydroxy cholesterol, known as D3 or vitamin D subcutaneously\(^{(12)}\).

Vitamin D plays an important role in bone building as well as a large cycle in many functions including immunosuppression and enzyme activation\(^{(13)}\).

In order to clarify the health effects that affect Cigarette and narghile smokers and because of the spread of the phenomenon of smoking narghile in Iraqi society, the current study aimed at studying the effect of smoking cigarettes and narghile on a number of blood variables and vitamin D. in males.

**Results**

**Concentration of vitamin D:**

The results of the current study presented a decrease in the concentration of vitamin D in the two groups of smokers of narghile and cigarette compared to the control group. The average vitamin D concentration in the serum of the three groups (30.9, 10.9, 13.3 ng / ml respectively) was significant in vitamin D concentration in the three studied groups at the probability level \(P <0.05\). Figure (1) shows the effect of smoking for Cigarette and narghile on the concentration of vitamin D in studied groups.

The least significant differences (LSD) showed significant differences in the concentration of vitamin D in the three studied groups \(P <0.05\).

**Complete Blood Count CBC**

**white blood cells WBCs count:**

The results of the current study presented an rise in the number of white blood cells in the two groups of smokers accorded to the control group. The average number of white blood cells in the control group and the group of cigarette smokers and the narghile smokers was \((7.1, 8.6, 8.9) \times 10^3\) cell / mm\(^3\).

The analysis of variance showed significant differences in WBCs count in the three studied groups at the probability level \(P <0.05\). Figure 7 shows the average number of white blood cells in the studied groups.

The LSD test showed significant differences in white blood cells count at \(P <0.05\).

**Red blood cells RBCs count:**

The results of the current study showed an increase in the number of red blood cells in the two groups of smokers accorded to the control group, with the average number of red blood cells in the control group and the group of cigarette smokers and narghile smokers \(5.3, 6.9, 7.0) \times 10^6 / \text{mm}^3\).

The results showed a significant difference in the number of red blood cells in the studied groups at the probability level \(P <0.05\). Figure 8 shows the average number of “red blood cells” in the studied groups.
Concentration of hemoglobin:

The results of the present study showed a high concentration of hemoglobin in the two groups of smokers compared to the control group with mean concentration of hemoglobin in the control group and the group of cigarette smokers and the group of narghile smokers (14.1, 15.2, 15.9) g / 100 ml, respectively.

The results of the variance analysis showed significant differences in mean hemoglobin concentration in the studied groups at the probability level (P <0.05). Figure 9 shows the average concentration of hemoglobin in the studied samples.

Mean Cell Volume (MCV).

The results of the current study showed an increase in the size of red blood cells in the two groups of smokers compared with the control group, with mean red blood cell count in the control group and the group of cigarette smokers and the group of narghile smokers (82.72, 88.56, 95.31) vimto liter respectively.

The results of the variance analysis presented significant differences in the mean values of the red blood cell volume (MCV) at the probability level (P <0.05). Figure 10 shows the average volume of the “red blood cell” in the studied groups.

Mean concentration of hemoglobin MCH:

The results of the present study showed an increase in the average concentration of hemoglobin in the two groups of smokers compared with the control group. The average concentration of hemoglobin in the control group and the group of cigarette smokers and the group of narghile smokers was 26.4, 38.6 and 40.5 pg / ml respectively.

The results of the variance analysis presented significant differences in mean hemoglobin concentration values in the studied groups at the probability level (P <0.05). Figure 11 shows the average concentration of hemoglobin in the studied groups.
Results of LSD showed significant differences in mean hemoglobin concentrations in the studied groups at the P <0.05).

Mean concentration of Hemoglobin Concentration MCHC:

The results of the current study presented significant differences in the mean values of the Hb concentration in the studied groups accorded to the control group. There was an increase in the values of the average concentration of Hb in the two groups of smokers accorded to the control group with the average concentration of Hb in the control group and the group of cigarette smokers and the group of narghile smokers was (31.5, 39.1, 38.5%) respectively.

The results of the variance analysis presented significant differences in the mean values of the serum hemoglobin concentration in the studied groups at the probability level (P <0.05). Figure 12.3 shows the average concentration of Hb in the studied groups.

Platelets Count (Plts):

The results of the current study presented an increase in the number of platelets in the group of smokers accorded to the control group. The average number of blood “platelets” in the control group and the group of cigarette smokers and the group of narghile smokers was 254000, 341000, 359000 / mm³, respectively.

The results of the variance analysis presented significant differences in mean blood platelet counts in the studied groups at the probability level (P <0.05). Figure 13-3 showed the average number of platelets in the studied groups.

Discussion

Smoking is a global problem. One of the leading causes of death is Cigarette smoking is. According to the “WHO” estimates that (2.4 billion) humans universal expended tobacco in burning, mastication, absorption or dropping. The “WHO” described that tobacco would be accountable for (10 million) deaths per year during the period 2020-2030, with 70% of deaths occurring in developing nations because of including at least 200 toxic substances and (80) known or supposed carcinogens, and large amounts of oxidants and free radicals that cause oxidative stress, lung oxidation and apoptosis14,14.

Cigarette smoking has most vital risk factors for atherosclerosis and increased deaths from chronic heart disease (CHD). Although the exact mechanism of the role of tobacco smoke in the atherosclerosis process is still not fully understood, many of the chemicals among the thousands present within tobacco smoke produce harmful and toxic effects on health (5).

There are many naturally occurring organelles in the cell that are rich in alkalis, which are tobacco and are rich in nicotine and some other toxic metals that have nicotine-like chemical activities in tobacco. These are arsenic, mercury, lead, cadmium, chromium, polonium and beryllium. The most important chemical components found in cigarettes are “tar”(3).

The most common way of “tobacco” consumption is Smoking, and the resulting vapors are inhaled. Risk substances (nicotine) are absorbed over and done with the pulmonary vesicles in the lungs and chemical reactions at the nerve endings, dopamine and epinephrine willfree, which are often associated with pleasure. Therefore, a large number of people continue to smoke in developing countries. Cigarette smoking is a known risk factor for many diseases such as the respiratory tract, heart, blood vessels and tumors, and also affects male fertility functions. Nicotine acts as a vasoconstriction, which means that the blood vessels become narrower and reduce blood flow. (15).

Smoking is a cause of cancer, heart disease, stroke and is also closely associated with stomach ulcers, (gum, sudden infant death, and metabolic) syndromes. Consumed of cigarette has a major adverse effect on various body systems especially on the “cardiovascular system”. Smoking in various forms is a major risk factor for “atherosclerosis” and “coronary heart disease”. (16).
Smoking is one of the most common and widespread addictive practices, affecting human behavior. Smoking is increasing rapidly throughout the developing global and is one of the greatest threats to present and future global health. Approximately (20%) of all “coronary heart disease” losses can be recognized to smoking. Cigarette smoking is an important risk factor for “coronary artery disease”, “arteriosclerosis” and disorders of peripheral vascular. (17).

Cigarette smoke is a risk factor for peripheral vascular disease, coronary arteries, and cerebral blood vessels. In general, a one to three-fold increase in the risk of MI was observed among current cigarette smokers.

Although smoking has a severe and chronic effect on blood standards, however, there is a lack of studies on the influence of cigarette smoking on blood in humans and animals. Over the earlier decade, it has been suggested that cigarette smoking has an effect on blood features and also leads to death. “White blood cells” has been detected nearly as high in smokers. There are some studies on the effect of smoking on platelets. In addition, data from several studies were not compared to non-smoking as control groups. Showed a high percentage of average volume of “MPV” in the smoker, which reduced after the smoker prevent from smoking. Though, no effect on “MPV” by smoking. In the light of this study, we compared the total number of “white blood cells” (WBC), “Platelets” (PLT), Platelet Crit (“PCT”), Average Platelet Size (“MPV”), Platelet Distribution (“PDW”) (P-LCR) for both smokers and non-smokers (14).

A strong correlation was found between cigarette smoking, arteriosclerosis and cardiovascular disease. Several studies have considered that endothelial injury is a major initial event in causing cardiovascular disorders. However, the exact pathophysiology of the harmful effects of smoking on the lining is not very clear. Potential mechanisms for smoking damage include increased thrombocytopenia and inflation, changes in lipid and lipoprotein levels, changes in hemostasis, increased enumeration and neutrophil activation (18).

In addition, smoking has been associated with the metabolism deformation of vitamin D. However, most studies have been conducted on the relationship between smoking and osteoporosis in postmenopausal women or elderly men after prolonged exposure to the toxic agent, and there is little information on the effect of smoking on bones in young people (10).

Vitamin D deficiency is common all over the global; even in areas close to the equator where it is assumed that exposure to sunlight is high. Furthermore to its known effects on calcium balance, “vitamin D” has a variety of functions, including immunological and anti-inflammatory effects. Many diseases have been related with “vitamin D” deficiency, including cancer and inflammatory diseases, although precise mechanisms are still under clarification. Previous epidemiological studies have shown a causal link between lung function and “vitamin D” levels. In lung-related lung diseases, lung destruction is partially mediated through inflammation, oxidative stress, and increased protease. Many of these processes are formed by “vitamin D”. Furthermore, studies in the laboratory and animal models suggest that cigarette smoke may affect with the anti-vitamin D effects, (19).

**Conflict of Interest:** There is no conflict of interest among the authors.

**Funding:** Self

**Ethical Clearance:** This study is ethically approved by the Institutional ethical Committee.

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Correlation between Hyperglycemia Stress and Short-Term Memory Function in Thrombotic Stroke Patients

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Abstract

Background: Hyperglycemic stress is associated with a disorder of the entire cognitive domain in the early phase of stroke, which is memory impairment. Acute changes in blood glucose are known to affect the cerebral blood flow.

Objectives: To determine the relationship between stress hyperglycemia with short-term memory function in thrombotic stroke patients.

Method: An observational analytic research with the case-control with a patient’s of 72-hour onset thrombotic stroke that went to the Emergency Unit and was admitted to the Neurology Department of Dr. Soetomo General Hospital from January to December 2014. 42 subjects were enrolled (21 subjects in the case group and 21 subjects in the control group). The sampling of the study was conducted according to the cases that came in succession until the achievement of the subject was determined.

Results: After bivariate and multivariate analysis, hyperglycemic stress had no significant correlation to memory function (p = 0.525) and OR 0.667 (CI95% = 0.190-2.334). There was a significant correlation between educational level and cognitive function (p = 0.003) also between hypertension and cognitive function (p = 0.019).

Conclusion: Stress Hyperglycaemia has no significant relationship with memory function. Education levels and hypertension have a significant correlation with memory function.

Keywords: Memory Function, Word List Memory Task, Hyperglycemic Stress

Introduction

A stroke is an acute-onset neurological deficit syndrome, which persists for more than 24 hours. A stroke involves the focal central nervous system and is the result of cerebral circulatory disorders ¹. In acute ischemic stroke, there is a sudden decrease or the loss of blood that flow to the brain resulting in a neurological deficit. Most of the causes are atherothrombosis of the intracranial artery or large artery in the neck, as well as the embolism of the heart ². Atherosclerosis is a pathological description of the most frequent vascular obstruction that causes a thrombotic stroke. Atherosclerotic plaque exist makes the blood flow to the area will decrease, it could even stop altogether. When the decreased blood flow to the minimal needs of the brain is unfulfilled, it will arise clinical symptoms ³.

Hyperglycemia is a common manifestation of critical illness and surgery. Hyperglycemia is caused by acute metabolic and hormonal changes that correlation with response to the stress and injury. Hyperglycemia is one of the important risk factors for stroke that found in ± 43% of stroke patients, either due to the hyperglycemia stress and diabetes mellitus (25%) ⁴.

Cognitive understanding is the process whereby all the sensory input (tactile, visual, auditory) will be altered,
processed, stored, and then used for perfect interneuron correlation, thus the individual is able to sense the sensory input. The cognitive domains or modalities according to Hodges consist of three modalities, namely: attention/concentration, memory, intellectual function, social behavior and personality. Memory is an individual process for storing and retrieving information.

Acute stroke causes cognitive impairment not only in the location of infarct tissue but also in areas adjacent to inadequately perfused lesions. Global cognitive impairment might include memory impairment, attention, executive function or visuoconstruction among other cognitive domains. Hyperglycemia is associated with the increased mortality and poor functional outcomes of post-stroke and might be a predictor of cognitive function output. Acute changes in the blood glucose are known to affect regional cerebral blood flow and cause osmotic changes in brain neurons.

**Method**

An observational analytic research with the case-control design was used in this study along with a patients of 72-hour onset thrombotic stroke that went to the Emergency Unit and was admitted to the Neurology Department of Dr. Soetomo General Hospital since January to December 2014. The subjects of the study were all thrombotic stroke patients treated in Seruni A, Seruni B, and Seruni Room and fulfilled inclusion and exclusion criteria that taken with consecutive sampling. The control group is a patient with normal Word List Memory Task results, while the case group is a patient with an abnormal Word List Memory Task. Clinical Data

Demographic Characteristics

The subjects were 42 people, consisting of 24 men (57.1%) and 18 women (42.9%). In the case group, the number of male subjects was 14 people (66.7%), while in the control group 10 people (47.6%). The female subjects in the case group were 7 (33.3%), while the control group was 11 people (52.4%). Gender has no significant correlation to cognitive function with p = 0.212 (Table 1). The mean age of the subjects in the case group was almost the same (50.05 ± 6.924 years), compared to the control group (51.05 ± 7.032 years) (p = 0.645), resulting no age difference was found to cognitive function (Table 1).

The education level in the case group consisted of 11 people (52.4%) graduated from elementary school, while 2 people (9.5%) in the control group. The level of Senior High School, Vocational High School, and College in the case group consisted of 10 people (47.6%), while 19 (90.5%) people in the control group. There was a significant correlation between educational level and cognitive function (p = 0.003) (Table 1).

Clinical Data

Subjects characteristics based on hypertension in the case group found that stage 1 hypertension were 10 people (47.6%), while in the control group were 3 (14.3). Then, Hypertension stage 2 in the case group were 11 people (52.4%), while in control group were 18 (85.7%). There was a significant correlation between hypertension and cognitive function (p = 0.019). Based on the risk factor of dyslipidemia, in the case group found that 11 people (52.4%) had dyslipidemia, while in control group were 10 (47.6%). Normal lipids subjects in the case group were 10 people (47.6%), while in the control group were 11 (52.4%). There was no significant association between dyslipidemia and cognitive function (p = 0.758) (Table 2). In the case group, 7 people (33%) had hyperglycemia stress and in the control group were 9 (42.9%), while in the case group unstressed hyperglycemia were 14 (66.7%), and in the control group were 12 (57.1%). Stress hyperglycemia have no significant correlation to cognitive function (p = 0.525).
Correlation of Odds Ratio (OR) between stress hyperglycemia and cognitive function was 0.667 with Confidence Interval (95% CI = 0.190-2.334), which means hyperglycemia stress to be 0.667 times more frequent for cognitive function impairment (protective factor), where it was obtained number 1 among Confidence Interval indicating that this relationship was not clinically significant (Table 3).

Table 1 Clinical Characteristics of Research Subjects

<table>
<thead>
<tr>
<th>Subject Variable</th>
<th>Group</th>
<th>Case n=21</th>
<th>Control n=21</th>
<th>Total n=42</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>Stage 1</td>
<td>10 (47.6%)</td>
<td>3 (14.3%)</td>
<td>13</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>Stage 2</td>
<td>11 (52.4%)</td>
<td>18 (85.7%)</td>
<td>29</td>
<td>0.758</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>Yes</td>
<td>11 (52.4%)</td>
<td>10 (47.6%)</td>
<td>21</td>
<td>0.525</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>10 (47.6%)</td>
<td>11 (52.4%)</td>
<td>21</td>
<td>0.758</td>
</tr>
<tr>
<td>Stress</td>
<td>Hyperglycemia</td>
<td>7 (33.3%)</td>
<td>9 (42.9%)</td>
<td>16</td>
<td>0.525</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>14 (66.7%)</td>
<td>12 (57.1%)</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2 Correlation between Hyperglycemia Stress with Cognitive Function

<table>
<thead>
<tr>
<th>Cognition Function</th>
<th>Case</th>
<th>Control</th>
<th>p</th>
<th>RO (IK 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Hyperglycemia Stress</td>
<td>Yes</td>
<td>7</td>
<td>33.3</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>14</td>
<td>66.7</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>100</td>
<td>21</td>
<td>100</td>
</tr>
</tbody>
</table>

Discussion

The sex ratio was 24 men (57.1%) and 18 woman (42.9%). This relevant to stroke epidemiologic data that men have more strokes experience than women. The mean age of the case group subjects was almost the same (50.05 ± 6.924 years) compared to the control group (51.05 ± 7.032 years). The mean age of the subjects was relatively young compared to the stated that the mean age was 65 ± 11.6 years. This study found a significant relationship between the level of education with cognitive function (p = 0.003). This was consistent with other studies suggest that education levels have an effect on memory.

A significant correlation was found between hypertension with cognitive function (p = 0.019). This is in accordance with research conducted by Tsao et.al that hypertension plays a role to decrease the cognitive function including memory. The changes in regional brain blood flow in hypertensive patients affect the cognitive function \(^ {11}\). Hypertension causes a direct consequence of demyelination due to hypoxia and ischemia and might lead to subcortical-cortical disconnection. There was
no significant correlation between dyslipidemia and cognitive function. This was consistent with studies suggest that there was no significant correlation between changes in plasma cholesterol levels and cognitive function 12.

Based on hyperglycemia risk factors, in the case group, fewer patients had hyperglycemia stress than the control group, but no significant relationship was found between hyperglycemia stress and cognitive function (p = 0.525). This not in accordance with the study conducted by Pan, which states that hyperglycemia stress was associated with impairment in all cognitive domains including the memory in cortical infarction, but this not found in subcortical infarction 13. This difference might due to this study that infarct stroke was not grouped based on its lesion location i.e., cortical and subcortical. Another cause was the age of the study subjects, in this study was ≤60 years whereas in other studies was <86 years old 14.

A study conducted by atkinson et,al states that 61% of patients that passed the acute period of stroke treatment and have a 1 or 0 NIHSS score encounter functional recovery well. But, the cognitive impairment was severe enough and only about 41% could return to work within 6 months after the stroke 15. However, it was found that the location of lesions and lesion sides did not affect the occurrence of cognitive function impairment, while the size of the lesions had an effect on the occurrence of cognitive dysfunction. Patients with high NIHSS scores are more likely to experience psychomotor speed disorders. In studies conducted Danovska M, the type and severity of cognitive impairment depend on the stroke picture. The location and size of the lesions also affect the occurrence of cognitive impairment in stroke 16.

Conclusion

There was no association between stress hyperglycemia with short-term memory function in patients with thrombotic stroke. Education levels and hypertension have a significant correlation with memory function. Further research using an observational analytic research method with a cohort design was needed, thus the results could be used to a better assessment of causal correlation.

Ethical Clearance: This research involves participants in the survey using sampling method that was accordant with the ethical research principle based on the regulation of research ethic committee. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, nonmaleficence, and justice.

Conflict of Interest: The authors swear that there is no conflict of interest related with this paper.

Source of Funding: This study is done with authors’ funding only.

Author’s Contribution

Muhammad Hamdan, Yudha Haryono, Abdulloh Machin, and Dian Puspitarini are doing the laboratory research, while Riani Wisnusono is the initiator of the research as well as the one who writes the report of laboratory result into this paper.

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The Difference between hs-CRP and Serum IL-6 Levels in Patients with Glaucoma and Non-Glaucoma

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Abstract

Background: Glaucoma is a group of eye diseases that have the characteristic of optic nerve damage with a specific pattern and field loss of view with or without increased intraocular pressure (IOP). The objective of the study was to analyze the association between glaucoma incidence with hs-CRP and IL-6 levels.

Method: This study used patients who fulfilled the inclusion criteria. Inclusion criteria were in the form of patients with a primary closed-angle diagnosis, willing to participate in the research. The study was conducted in the period from March to August 2013. The research procedure included patient examination, hsCRP and IL-6 level examination.

Results: Mann-Whitney test result showed difference of hs-CRP with p value of 0.755 and difference of IL-6 level with p = 0.95. Thus, there was no significant difference in the levels of hs-CRP and IL-6 in patients with glaucoma and non-glaucoma.

Conclusion: Hs-CRP and serum IL-6 levels in the glaucoma group did not differ from the non-glaucoma group.

Keywords: hs-Crp, IL-6, glaucoma, intraocular

Introduction

Glaucoma is a group of eye diseases that have the characteristic of optic nerve damage with a specific pattern and field loss of view with or without increased intraocular pressure (IOP). WHO States that glaucoma is the second rank as the cause of irreversible blindness in the world. Primary closed-angle glaucoma (PCAG) is the most common form of glaucoma in Asian countries. A typical PCAG in European countries is an acute attack, unlike Asian and African countries that tend to develop gradually as chronically closed angle asymptomatic.

Glaucoma disease affects more than 66 million individuals worldwide, causing bilateral blindness at 6.8 million. It is estimated that 3.9 million individuals became blinded by PCAG in 2010. By 2020, this number is assumed to increase to 5.3 million. Eighty-six percent of PCAG patients are in Asia, with an estimated 48.0% in China, 23.9% in India and 14.1% in Southeast Asia. Indonesia has the highest number of blindness in Southeast Asia. From the 1993-1996 health survey, glaucoma caused 13.4% of blindness after cataracts. Mimiwati et al. found chronic PCAG (58.5%) was the most common form followed by subacute (26.8%) and acute group (14.6%).

Glaucoma risk factors such as old age increase the percentage of closed angle and increase the incidence of PCAG. Women are at risk 3 to 4 times compared to men because they are related to the fact that they are gonioscopy which has a shallower eye angle than men. Other risk factors for increased IOP are family history, severe myopia, systemic hypertension, cardiovascular disease, migraine headaches, peripheral vasospasm and previous neurological damage. Acute attacks of closed-angle glaucoma lead to blindness within hours or days, in contrast to primary open-angle glaucoma that is slow

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and gradual loss of vision. In addition, the other most important things are the speed and the accuracy of the management. The accurate diagnosis in the form of intermittent and chronic of PCAG is also important because prophylactic treatment can protect the eyes from acute attacks and prevent damage due to repeated intermittent attacks or chronic closed angle. Loss of vision due to glaucoma is irreversible; however, it can be treated if the diagnosis is established at an early stage. Many individuals with glaucoma become blind because they have not received good help 3.

Retinal ganglion cell (RGC) apoptosis is a key of neurodegenerative glaucoma, but the detail of the underlying process remains unclear. Various mechanisms have been found such as the deprivation of neurotrophic factors, hypoperfusion or ischaemia, glial cell activation, glutamic excitotoxicity and abnormal immune responses which lead to an end result of RGC apoptosis. The in vivo study explains the role of an adaptive immune response in the pathogenesis of glaucoma. Increased IOP and ischemia is a stress that acts as an immunostimulator signal, triggering an activation of an immune response that was initially useful for repairing tissue 4.

IL-6 is a pleitropic cytokine, which both uses a common signal transduction component with other IL-6 family members. A specific IL-6 signal involves the interaction of gp130 with IL-6 alpha receptor present in the membrane (IL-6Ra). Research conducted in vivo and in vitro showed elevated levels of T helper (Th2) lymphocyte cell cytokines which are IL-6 and Th1 cytokines including interferon γ (IFN-γ), IL-1, IL-2, IL-17 and tumor necrosis factor α (TNF-α) associated with retinal damage and RGC. The increase in intraocular IL-6 levels has not been shown to affect serum IL-6 levels 5.

Serum IL-6 examination is influenced by several conditions because these cytokines are produced by various cells after stimulation of infection, trauma, immunological reactions and other systemic inflammation. In addition, IL-6 examination is still not conducted in the clinical laboratory; thus, it needs a practical examination. High-sensitivity C-reactive Protein (hs-CRP) is a solution to this problem, since it is useful in filtering out inflammatory markers such as infection, trauma, immunological reactions and systemic inflammation. Its production in the liver is induced directly by IL-6. Hs-CRP examination has grown rapidly as predicted risk of coronary heart disease, stroke and other peripheral vascular diseases; thus, it needs to be evaluated its potential for the diagnosis of glaucoma 6.

Method

This research is a laboratory analysis research with cross sectional design. The inclusion criteria of respondents were patients with a primary closed-angle diagnosis and willing to participate in the study. While the exclusion criteria were patients with coronary heart disease, systemic infections, viral conjunctivitis, and diabetes mellitus. The sampling technique used simple random sampling. The research procedure included patient examination, hsCRP examination, and examination of IL-6 levels 7.

Examination of IL-6 levels was conducted by using ELISA method. This examination used the ELISA microreader tool from R&D Systems. The venous blood sample from the cubital vein had previously been disinfected with 70% alcohol, then venous blood was inserted into a 2 ml plain vacutainer tube. Each tube was labeled (patient name, date and time of sampling, and the initials of the subjects taking the sample). Samples were incubated for 30 min and then centrifuged 3000 rpm, 15 min to separate serum. The serum was put into several microcentrifuge tubes 8. The tube was labeled and immediately shipped to the Bank Jaringan, stored in the -70°C freezer until further inspection. The retrieval time to sample storage was less than 1 hour 9. All collected data were arranged in tabular form and processed statistically. The statistical test used to determine the difference of serum hs-CRP and IL-6 levels in glaucoma and non-glaucoma patients was Mann-Whitney test with p <0.05 10.

Results

Examination of hs-CRP levels in study subjects’ serum showed that the mean value of hs-CRP in glaucoma patients was not different compared to non-glaucoma control hs-CRP values. The mean values in the control group tend to be larger and the standard deviation was also greater than the mean glaucoma group. The range of variation in hs-CRP values was also significant in the glaucoma group from 0.084 to 5.78 mg/L while in the non-glaucoma control group it was greater than 0.001 to 7.934 mg/L and the median values for each group were 1.362 and 1.710 (Table 1).

IL-6 examination results showed no significant difference in mean values in both groups of study
subjects. The mean value of IL-6 glaucoma group was greater than non glaucoma. The mean rate of IL-6 levels in the glaucoma group was greater than non glaucoma but the mean level of hs-CRP in the glaucoma group was smaller than non glaucoma. This result was not in accordance with physiological conditions, ie when the level of IL-6 increased, it will be followed by increased levels of hs-CRP. Standard deviations in both groups were also relatively large even it was larger in non glaucoma group. The range of IL-6 levels was also high in the glaucoma group from 0.21 to 9.1 pg/ml and the non-glaucoma group from 0.16 to 9.63 pg/ml with median values of 1.910 and 0.775, respectively (Table 1).

Test of normality of difference of hs-CRP level between glaucoma and non glaucoma patient has significance <0.05. Normality test with Kolmogorov-Smirnov on glaucoma group showed p value = 0.004 was in non glaucoma group p = 0.001. The situation showed abnormal data distribution (Table 3). The normality test of difference of IL-6 level between glaucoma and non glaucoma patient had significance <0.05. Test of data normality with Kolmogorov-Smirnov in glaucoma group showed p value = 0.106 was in non glaucoma group p = 0.002. Although there was a value of p >0.05, it remains statistical tests for abnormal data distribution because another p value <0.05 (Table 2).

The normal distribution curve was shown at the level of IL-6 glaucoma which was symmetrically pointed, although the median value slightly shifts toward the left of 1.910 shifts from the mean of 2.319. Non-glaucoma group distribution curve was not normal because the median value of 0.775 shifts away from the mean value of 1828; thus, the curve figure is not symmetrical. The pattern of non-glaucoma group population was more to the right of the median region. The results of normality test of hs-CRP and IL-6 results in glaucoma patients and non glaucoma patients with Kolmogorov-Smirnov showed mostly p <0.05. The value in the normality test of IL-6 data in glaucoma patients with Kolmogorov-Smirnov p >0.05 was p = 0.106 and overall data distribution remained abnormal. Mann-Whitney test result difference of hs-CRP with p value of 0.755 and difference of IL-6 level with p = 0.95. Thus, there was no significant difference in the levels of hs-CRP and IL-6 in glaucoma and non-glaucoma individuals (Table 3).

Discussion

The results of this study have stated that there was no difference in hs-CRP levels in glaucoma and non glaucoma. The results were similar to previous studies; thus, the hs-CRP examination had no clinical significance in the diagnosis of glaucoma. Serum CRP examination is routine in clinical laboratories as a marker of various acute and chronic inflammatory diseases. The production of CRP in hepatic cells are stimulated various proinflammatory cytokines such as IL-1, IL-6, TNF-α, IFN-γ and TGF-β. Small amounts are also produced locally in lymphocytes, and vascular wall muscle tissue in patients with coronary heart disease. CRP production increases in the inflammatory process. The normal range of CRP values in adult individuals ranges from 1.5 mg/L for males and 2.5 for females.

IL-6 is a single protein cytokine not only in the production of lymphoid and non-lymphoid cells. IL-6 is also expressed by normal or transformed cells such as macrophages, fibroblasts, synovial cells, endothelial cells, glial cells, keratocytes and tumor cells. IL-6 expression induced by various stimuli includes cytokines such as IL-1, TNF-α, PDGF, bacterial and viral infections and microbial components such as lipopolysaccharide. Its role has been well known in the minimal chronic inflammatory state of obesity, diabetes, cardiovascular disease as well as in infections and sepsis. In healthy individuals independent of inflammatory reactions, the IL-6 concentrations are very low from 0.2 to 7.8 pg/ml and can rise to a high of 1600 pg/ml concentrations in sepsis.

IL-6 in the systemic circulation is a pleiotropic cytokine involved in the regulation of the immune system, acute phase reaction and hematopoesis. IL-6 is associated with an acute phase reaction functionally referred to as hepatocyte stimulating factor (HSF) because it stimulates hepatocytes in producing CRP. The mean rate of IL-6 group of glaucoma was greater than non glaucoma, but the mean hs-CRP level in the glaucoma group was smaller than non glaucoma.

This result is not compatible with physiological conditions, ie when the level of IL-6 increases, it will be followed by increased levels of hs-CRP. This suggests that no significant difference in IL-6 levels in the glaucoma and non glaucoma groups needs to be analyzed further. The results of IL-6 studies on serum or systemic circulation do not coincide with IL-6 studies in aqueous humour can be influenced by various factors. Intraocular IL-6 cytokine levels are too minimal to affect levels in the systemic circulation; thus, the threshold
levels of IL-6 glaucoma and non glaucoma are very narrow. The intraocular immune privilege mechanism also limits the local inflammatory process to not spread to the circulation; thus, the levels of IL-6 in the glaucoma group are not different from that of non glaucoma 18.

**Conclusion**

Based on the results of research, it can be concluded that serum hs-CRP and IL-6 levels in the glaucoma group did not differ from the non glaucoma group. Serum hs-CRP testing can not be utilized in assisting clinical diagnosis of glaucoma, whereas serum IL-6 examination also has not been able to assist in clinical diagnosis of glaucoma.

**Ethical Clearance** : This research process involves participants in the survey using a questionnaire that was accordant with the ethical research principle based on the regulation of research ethic committee. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, nonmaleficence, and justice.

**Conflict of Interest** : The authors swear that there is no conflict of interest related with this paper so far and in the future.

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The Risk Factors Effect of Knee Osteoarthritis Towards Postural Lateral Sway

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Abstract

Background: Osteoarthritis (OA) is the main cause of impairment and disability in elderly. Patient with OA experience progressive functional impairment shown in increasing dependence on walking, using stairs and etc. Patients with OA experience increased static postural sway in the lateral and anteroposterior due to several factors.

Objective: This study aims to analyze the risk factors of knee osteoarthritis on postural lateral sway.

Method: This research is a cross sectional study. The severity of the respondents’ knee OA, unilateral/bilateral, dominant side, knee joint alignment, body mass index (BMI), and postural lateral sway were measured with Gait Analyzer Peak Motus 2000, and their quadriceps muscle activity was assessed with Myomed. Respondents involved in this study were 38 elderly with unilateral or bilateral knee OA stage 1 until 3 based on the criteria of Kellgren & Lawrence. The data were analyzed with multiple linear regression.

Results: The results showed that postural lateral sway was 2.23±0.59 cm. BMI, dominant side, OA types, OA severity, Quadriceps angle, and tibio-fibular angle (TFA) were found with the p value = 0.421, 0.599, 0.780, 0.913, 0.596, and 0.984 respectively. The Visual Analogue Scale (VAS) variable was R Square = 0.291, p = 0.001, Y = 0.3096 and X = 0.9393.

Conclusion: Pain is the strongest predictor factor of postural lateral sway disturbances in patients with knee OA.

Keywords: osteoarthritis, postural lateral sway, pain.

Introduction

Osteoarthritis (OA) is known as degenerative joint disease that is considered to be the most common form of arthritis. The characteristics of OA include joint and bone damage followed by the formation of new bone on the joint surface. The common types of joints that tend to experience OA are knee, pelvis, hand, and spine joint. The incidence of knee OA is 240 per 100,000 individuals per year. In Indonesia, the prevalence of osteoarthritis is 5% in individuals aged <40 years old, 30% in individuals aged 40-60 years old, and 65% in individuals aged >61 years old. The prevalence of knee OA is deemed high, which is 15.5% in males and 12.7% in females. The risk factors of OA are genetic, age, gender, ethnicity, obesity, occupation, muscle weakness, and biomechanics.

The elderly patients with OA will experience an increased postural sway if the gravitation center shifts from the center of the body. Proprioceptive suboptimal limbs cause the increase of the received force to be transmitted to the hip and knee. If it repetitively happens, the force triggers the occurrence of OA. Patients with knee OA encounter increased static postural sway in the lateral and anteroposterior, decreased knee proprioception, and decreased maximal voluntary contraction and quadriceps muscle activation.
compared to control group in the same age group. The most influential factor of OA in postural sway is the severity of pain\textsuperscript{5,6}. Other factors are age, gender, knee joint stiffness, and maximal walking speed\textsuperscript{7}.

This study intended to investigate the factors that are related to the knee OA cases such as the severity of knee OA, unilateral and bilateral knee OA, the dominant side of knee, knee joint alignment (TFA and Q angle), BMI, pain, and quadriceps muscle activity towards the incidence of postural sway in patients with knee OA.

**Method**

This study applied cross sectional and observational analytic design and was conducted on January until April 2012, in which the sample was collected using consecutive sampling. The criteria of knee osteoarthritis diagnosis are based on the guidelines set by the American College of Rheumatology (ACR). There were 38 samples that fulfilled the inclusion criteria including: (1) Patients with unilateral or bilateral knee OA stage 1 until 3 based on the criteria of Kellgren & Lawrence; (2) Aged 40-65 years old; (3) No limited range of motion of the lower limb member joints; (4) understand and follow the examination conditions; (5) Able to ambulate independently without ambulatory aids; (6) willing to participate in the study.

Measurements on the knee OA severity to decide whether it was unilateral or bilateral, dominant side of the body, knee joint alignment, and body mass index were conducted using Gait Analyzer Peak Motus 2000 to measure postural lateral sway while the quadriceps muscle activity was measured by Myomed equipment. The data were analyzed with multiple linear regression by using SPSS program for Windows (SPSS, Chicago, IL, USA).

**Results**

The result of sample measurement in this study revealed that knee OA mostly occurred in 41-65 year-old patients and the average stage was 58.03 years old and the SD was 5.635. The postural sway in the subjects was measured by using the equipment of Gait Analyzer with Peak Motus 2000 program. The measurement of postural sway was in the form of shifting center of body mass toward lateral (Table 1). The postural lateral sway was 1.25 cm until 3.37 cm with the average of 2.23 cm and SD of 0.59. The results of Kolmogorov–Smirnov test found the significance value of 0.468. The data of postural sway was considered normally distributed. Afterwards, the regression test was performed.

**Table 1. The Result of Sample Measurements**

<table>
<thead>
<tr>
<th>Descriptions</th>
<th>Results</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>41-65</td>
<td>58.03</td>
<td>5.635</td>
</tr>
<tr>
<td>Postural sway (cm)</td>
<td>1.25-3.37</td>
<td>2.23</td>
<td>0.59</td>
</tr>
<tr>
<td>Body Mass Index (kg/m2)</td>
<td>19.14-34.17</td>
<td>26.7</td>
<td>3.728</td>
</tr>
<tr>
<td>Visual Analogue Scale</td>
<td>3-7</td>
<td>4.16</td>
<td>1.027</td>
</tr>
<tr>
<td>Quadriceps Angle (o)</td>
<td>5-20</td>
<td>13.55</td>
<td>4.032</td>
</tr>
<tr>
<td>Tibio-femoral angle (o)</td>
<td>160-190</td>
<td>172.97</td>
<td>6.883</td>
</tr>
<tr>
<td>Max Vastus medial scale amplitude</td>
<td>36-400</td>
<td>95.39</td>
<td>60.102</td>
</tr>
<tr>
<td>Max vastus lateralis amplitude</td>
<td>45-307</td>
<td>94.03</td>
<td>51.78</td>
</tr>
<tr>
<td>Average vastus medial oblique amplitude</td>
<td>12-114</td>
<td>43.68</td>
<td>17.996</td>
</tr>
</tbody>
</table>
The result of pain and postural sway measurement based gender and OA characteristics with $p = 0.05$ showed statistically insignificant variable (Table 2). Most samples (33 individuals or 86.8%) are categorized in the overweight and obesity group based on the Asian scale of body mass index (Table 3). Another study regarding OA in Indonesia revealed that most OA patients (74.7%) had BMI more than 23, which is considered to be overweight. To add, a study conducted in Malaysia showed that people with obesity likely experience osteoarthritis.

**Table 2. The result of pain and postural sway measurement based on gender and knee OA characteristics**

<table>
<thead>
<tr>
<th>Descriptions</th>
<th>Average VAS</th>
<th>Average postural sway</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>4.3</td>
<td>2.75 cm</td>
<td>0.016</td>
</tr>
<tr>
<td>Female</td>
<td>4.1</td>
<td>2.13 cm</td>
<td></td>
</tr>
<tr>
<td>Osteoarthritis types</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unilateral</td>
<td>4.18</td>
<td>2.19 cm</td>
<td>0.857</td>
</tr>
<tr>
<td>Bilateral</td>
<td>4.15</td>
<td>2.24 cm</td>
<td></td>
</tr>
<tr>
<td>Osteoarthritis sides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dominant</td>
<td>4.1</td>
<td>2.21 cm</td>
<td>0.628</td>
</tr>
<tr>
<td>Non-dominant</td>
<td>4.2</td>
<td>2.35 cm</td>
<td></td>
</tr>
<tr>
<td>Osteoarthritis severity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 1</td>
<td>4.5</td>
<td>2.09 cm</td>
<td>0.863</td>
</tr>
<tr>
<td>Stage 2</td>
<td>3.9</td>
<td>2.18 cm</td>
<td></td>
</tr>
<tr>
<td>Stage 3</td>
<td>4.3</td>
<td>2.27 cm</td>
<td></td>
</tr>
</tbody>
</table>

The coefficient table (Table 4) was drawn to discern the role of each predictor (beta) and see if any predictor was significant in predicting DV (t value). Afterwards, the multiple linear regression analysis was conducted based on the method. According to pain variable, VAS had the square R value of 0.291 and the significance value of 0.0001. It can be seen that the variable was significant in predicting the postural sway (table 4).
### Table 3. The result of VAS and postural sway measurement based on body mass index

<table>
<thead>
<tr>
<th>Body Mass Index</th>
<th>N</th>
<th>Average Visual Analogue Scale</th>
<th>Average postural sway</th>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>5</td>
<td>4</td>
<td>1.79 cm</td>
<td></td>
</tr>
<tr>
<td>Overweight</td>
<td>9</td>
<td>3.7</td>
<td>2.23 cm</td>
<td>$F = 1.707$</td>
</tr>
<tr>
<td>Obese</td>
<td>24</td>
<td>4.3</td>
<td>2.31 cm</td>
<td>Sig = 0.196</td>
</tr>
</tbody>
</table>

### Table 4. The effect of variables in predicting the value of postural sway

<table>
<thead>
<tr>
<th>Variables</th>
<th>Beta</th>
<th>t</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.264</td>
<td>0.821</td>
<td>0.421</td>
</tr>
<tr>
<td>Dominant side of Osteoarthritis</td>
<td>-0.142</td>
<td>-0.534</td>
<td>0.599</td>
</tr>
<tr>
<td>Osteoarthritis types</td>
<td>-0.075</td>
<td>-0.283</td>
<td>0.780</td>
</tr>
<tr>
<td>Osteoarthritis severity</td>
<td>0.021</td>
<td>0.111</td>
<td>0.913</td>
</tr>
<tr>
<td>Visual Analogue Scale</td>
<td>0.376</td>
<td>1.766</td>
<td>0.092</td>
</tr>
<tr>
<td>Quadriceps Angle</td>
<td>0.099</td>
<td>0.539</td>
<td>0.596</td>
</tr>
<tr>
<td>Tibio-femoral angle</td>
<td>-0.004</td>
<td>-0.020</td>
<td>0.984</td>
</tr>
<tr>
<td>Vastus medial oblique - vastus lateralis ratio</td>
<td>1.783</td>
<td>0.360</td>
<td>0.722</td>
</tr>
<tr>
<td>Different latent of Vastus medial oblique - vastus lateralis</td>
<td>-0.208</td>
<td>-0.956</td>
<td>0.350</td>
</tr>
<tr>
<td>Stepwise method</td>
<td>Criteria 0.05 – 0.1 → VAS</td>
<td>R Square = 0.291</td>
<td>Sig 0.0001</td>
</tr>
</tbody>
</table>

### Discussions

From the result of the multiple linear regressions based on the enter method, it was revealed that the pain scale is the most influential factor towards the increase of postural sway in the study sample. The pain can alter the patient’s walking pattern, in which there is an unequal gait parameter between the pain in the form of a shorter stance phase. The restriction of joint motion and less muscle contraction in the location of the pain can affect the magnitude of the postural lateral sway. Another variable that was investigated in the study showed the significant result with the $p$ value of $p = 0.238$; however, it has a pivotal role in the case of postural sway.

This study found that the postural sway value ranged from 1.25-3.37 cm, with the average value of 2.23 cm. The observation in normal subjects using similar equipment and measurement method showed that postural lateral sway ranged from 1-2.5 cm with the average of 1.86 cm. In the comparison among normal individuals, there was a statistically significant difference measured by t test ($p = 0.03$). Another study in Indonesia revealed that the average postural sway in knee OA patients using posturography equipment was 2.32 cm. Despite employing different equipment, the result was quite similar with our study with the average postural lateral sway of 2.23 cm. This is due to the similarity between the basic characteristics of the study sample. Body mass index had weak positive correlation in predicting the postural sway with the correlation value of 0.112.
The femoral shaft walks slightly to the medial towards the knee. The oblique orientation occurred due to the inclination angle of 125° in femoral proximal. The joints of the proximal tibia are horizontally oriented; thus, the angle is formed (tibio-femoral angle/TFA) from the lateral side of 170 until 175°. The large angle formed by the pull of quadriceps is called a quadriceps angle or Q-angle. This angle is formed by (1) a line describing the resultant force of the quadriceps, formed by the point on the anterior superior iliac spine (SIAS) to the midpoint of the patella, and (2) the line connecting the tibia tuberosity and the center line of the patella 11. The increase of TFA and femoral anteverision angle is a predictor factor for the increase of Q-angle 12. The knee joint efficacy assessed with Q angle and TFA did not show any significant results in this study to predict the increase of postural sway with p = 0.596 for Q angle and p = 0.984 for TFA. This result is possible because of the relatively normal knee joint sessions in the study sample, in which the mean Q angle of 13.55 and the mean TFA of 172.97 are included in the normal range.

In other studies observing postural sway isometrically, it revealed that the activation and quadriceps muscle strength had significant correlation with the increased postural sway cases in patients with knee OA 13. However, different results of other studies are because most patients have obtained muscle quadriceps exercise program; thus, the activity of quadriceps muscle is improved. It can be discerned from the measurement results: the average VMO amplitude ratio towards VL was 110.02 and the average difference of VMO muscle latency towards VL was 0.037 second.

Due to the limitation of the sample with OA stage I, there is a possibility in error on the withdrawal of conclusions from the statistical analysis. Furthermore, there was no measurement in patients’ balance and questionnaire regarding the functional condition. Therefore, the effect of postural sway towards functional condition and patients’ balance in daily activity cannot be investigated.

**Conclusion**

Pain is the most influence of postural lateral sway in patients with knee OA. Other variables such as body mass index, OA types (unilateral/bilateral), dominant side of the limb with knee OA, knee joint, and quadriceps muscle activity have weak correlations and have no statistically significant effect.

**Ethical Clearance:** The study protocol was approved by the Ethical Commission to conduct basic science/clinical research in Dr. Soetomo General Hospital Surabaya, Indonesia. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, non-maleficence, and justice.

**Conflict of Interest:** The author reports no conflict of interest of this work.

**Source of Funding:** This study is done with individual funding.

**References**

predisposition of patients to knee osteoarthritis. Obes Med. 2019 Dec;16(September):100143.


Microbial Risk Assessment (MRA) As a Method of Assessment for Drinking Water Refill in Pattinggaloang District of Makassar City

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²Lecturer in Departement of Environmental Health University Moslem of Indonesia

Abstract

Refill drinking water is one of the answers to meeting the needs of Indonesian drinking water that is cheap and practical. This is the reason why people choose AMIU to be consumed. The purpose of this study is to determine the risk of microbial drinking water in the community consumed in the working area of Pattinggaloang Public Health Center in Makassar in 2019.

This type of research is descriptive with Microbial Risk Assessment (MRA) assessment on refill drinking water. The sampling technique was done by a total of 6 depot drinking water refill sampling. Data processing is done by the SPPS method, the data presentation is carried out using tables accompanied by narration.

Laboratory results carried out on 6 samples of refill drinking water obtained were as many as 6 samples which were stated to be Positive (+) containing bacteria. Coli. Based on a Quantitative Risk Assessment, it was concluded that from 6 DAMIU samples had a high risk concentration, for the estimated number of bacteria E. Coli in Refill Drinking Water in the surrounding area of the Pattinggaloang Health Center in Makassar City.

It is recommended for consumers to refill drinking water in cooking first, because pollution / contamination can occur starting from the process of taking raw water, processing and packaging / filling in gallons.

Keywords: Drink water, MRA, E.coli, Microbial risk

Introduction

WHO (2015) found that 663 million people still had difficulty accessing clean water. In connection with this water crisis, it is predicted that in 2025 nearly two-thirds of the world’s population will live in areas that experience water shortages. The forecast was reported by the World Water Assessment Program (WWAP), formed by the United Nations Educational, Scientific, and Cultural Organization Regarding Indonesia, in 2012. The World Water Assessment Program (WWAP) has recorded that Indonesia was ranked the worst in the service of the availability of clean water and suitable for consumption in Southeast Asia. One of the studies in Microbiology is the material for growing and growing microorganisms from the environment, including microorganisms that have an environment, one of which is in food. Food is one of the basic needs for human life. Food functions to maintain the body’s process of growth or development and replace damaged body tissues, obtain energy to carry out daily activities, regulate metabolism and various water, mineral and other body fluids, also plays a role in the body’s defense mechanism against various diseases.

According to WHO Risk assessment is a structured process for determining risks associated with all types of hazards - biological, chemical or physical in food. It has the aim of characterizing the nature and possible dangers resulting from human exposure to agents in food. Risk characterization usually contains qualitative and quantitative information and is associated with a certain degree of scientific uncertainty.

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Microbial Risk Assessment (MRA) is a methodology used to organize and analyze scientific information to estimate the probability and severity of an adverse event. Set on microbial food safety, this methodology can also help identify the stages in the manufacture, distribution, handling, and consumption of food that contribute to the increased risk of foodborne illness, and help focus resources and efforts to most effectively reduce the risk of foodborne pathogen.

Based on the Unicef Joint Monitoring report, the performance of the water and sanitation sector in Indonesia is still considered low compared to other countries in Southeast Asia. Of Indonesia’s population of around 218 million people in 2015, an estimated 103 million people (47%) did not have access to sanitation and around 47 million people (22%) did not have access to clean water. Only around 50% of the entire population of Indonesia has access to drinking water. Meeting the current needs of community drinking water varies greatly. Population needs for drinking water can be met through water served by piping systems (PAM), bottled drinking water (AMDK), and refill drinking water (AMIU). The tendency of the population to consume ready to use drinking water is very large so that the effort to replenish drinking water is growing very rapidly.

Makassar City Health Office shows the distribution of diarrhea cases according to Makassar city health centers in 2017 there were 46 health centers. The highest case of diarrhea according to the Makassar City Health Center in 2017 was the Pattingaloang Health Center with a diarrhea case of 933 cases. In the Pattingaloang Puskesmas working area there are 6 refill drinking water depots.

This is because careless disposal of human and animal feces will cause pathogens in the feces to transmit through the soil media and spread in water sources which are then used by humans. As a result, almost every year sanitation and contaminated drinking water contribute to 88% of child deaths due to diarrhea throughout the world. Based on this, the high incidence of diarrhea in infants in Indonesia can indicate poor sanitation and drinking water sources that are used by the community.

**Material and Method**

This type of research is descriptive with Microbial Risk Assessment (MRA) assessment on refill drinking water, the sampling technique is carried out in total total sampling of 6 refill drinking water depots. Collecting data using observation sheets, and using a questionnaire then carried out examination of drinking water samples in an integrated laboratory to FKM UMI. Data processing is done by the SPPS method, the data presentation is carried out using tables accompanied by narration. Inspection of Samples for drinking water that has been taken is done by checking the presence of E. coli in it. Tests are carried out three times each for the same sample of 6 samples.

**Materials and Tools**

The tools used in laboratory tests are: Incubator, Autoclaves, Weighing tools, Pipettes, Warp tubes, Tubes of dhamham, Cotton, Wire loops (ose) made of chromium-platinum, Burning bunsen Test tube racks. The materials used: Aquades, Lactose Broth (LB), Brilliant Green Lactose Broth (BGLB) 2%, Label paper, Aluminum foil, Drinking water samples.

**Result**

Based on table 1 shows the number of E. coli in the AMIU depot in the Pattinggaloan Puskesmas.

<table>
<thead>
<tr>
<th>code Sample</th>
<th>Cultur Bacteri</th>
<th>Result</th>
<th>Ket</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP 1</td>
<td>Positif (+)</td>
<td>9</td>
<td>Not Eligible</td>
</tr>
<tr>
<td>DP2</td>
<td>Positif (+)</td>
<td>23</td>
<td>Not Eligible</td>
</tr>
<tr>
<td>DP 3</td>
<td>Positif (+)</td>
<td>&gt;1400</td>
<td>Not Eligible</td>
</tr>
<tr>
<td>DP 4</td>
<td>Positif (+)</td>
<td>28</td>
<td>Not Eligible</td>
</tr>
<tr>
<td>DP 5</td>
<td>Positif (+)</td>
<td>7</td>
<td>Not Eligible</td>
</tr>
<tr>
<td>DP 6</td>
<td>Positif (+)</td>
<td>7</td>
<td>Not Eligible</td>
</tr>
</tbody>
</table>

Based on table 1 shows that of the 6 samples tested in the laboratory were positive (+) containing E. coli bacteria, refill drinking water that contained the highest bacteria in refill drinking water with DP3 code with the number of bacteria>1400 E. coli bacteria.
Table 2: Physical Quality Water refill drinking water depots in the working area of the Pattingaloang Community Health Center in Makassar City

<table>
<thead>
<tr>
<th>code Sampel</th>
<th>Suhu °C</th>
<th>pH Information</th>
<th>Turbidity Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depot 1</td>
<td>25</td>
<td>Eligible 6.03</td>
<td>Not Eligible 0.00</td>
</tr>
<tr>
<td>Depot 2</td>
<td>25</td>
<td>Eligible 5.65</td>
<td>Not Eligible 0.00</td>
</tr>
<tr>
<td>Depot 3</td>
<td>25</td>
<td>Eligible 5.43</td>
<td>Not Eligible 0.00</td>
</tr>
<tr>
<td>Depot 4</td>
<td>25</td>
<td>Eligible 5.8</td>
<td>Not Eligible 0.00</td>
</tr>
<tr>
<td>Depot 5</td>
<td>25</td>
<td>Eligible 5.86</td>
<td>Not Eligible 0.00</td>
</tr>
<tr>
<td>Depot 6</td>
<td>25</td>
<td>Eligible 5.88</td>
<td>Not Eligible 0.00</td>
</tr>
</tbody>
</table>

Based on table 2 shows that the frequency of distribution of respondents based on the physical quality of refill drinking water depot that meets the requirements with 6 samples Smelling parameters, turbidity parameters meet the requirements of 6 samples, temperature parameters meet the requirements of 6 samples, while those that do not meet the requirements namely parameters pH with a total of 6 samples on the physical quality of water.

Table 3: Estimation of Community Microbial Risk Assessments Consuming Refill drinking water in the working area of the Pattingaloang Health Center in Makassar City

<table>
<thead>
<tr>
<th>code sample</th>
<th>Cr (AMIU quality)</th>
<th>Cd (Konsentration bacteri)</th>
<th>(d) Dosis</th>
<th>Pinf.d (Infeksi/day)</th>
<th>Pinf.y (Infeksi/yaers)</th>
<th>Pill</th>
<th>categori</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP1</td>
<td>9</td>
<td>42.084</td>
<td>0.0042</td>
<td>9.6721E-06</td>
<td>4.1803E-03</td>
<td>1.636E-05</td>
<td>High risk</td>
</tr>
<tr>
<td>DP2</td>
<td>23</td>
<td>103.776</td>
<td>0.0103</td>
<td>5.2340E-05</td>
<td>1.9104E-05</td>
<td>3.653E-05</td>
<td>High risk</td>
</tr>
<tr>
<td>DP3</td>
<td>1400</td>
<td>7116.2</td>
<td>0.0007116</td>
<td>3.6163E-06</td>
<td>1.3199E-03</td>
<td>3.261E-05</td>
<td>High risk</td>
</tr>
<tr>
<td>DP4</td>
<td>28</td>
<td>154.7</td>
<td>0.0154</td>
<td>7.8523E-05</td>
<td>2.8562E-03</td>
<td>7.614E-05</td>
<td>High risk</td>
</tr>
<tr>
<td>DP5</td>
<td>7</td>
<td>31.535</td>
<td>0.0031</td>
<td>1.5753E-08</td>
<td>5.7501E-05</td>
<td>2.739E-05</td>
<td>High risk</td>
</tr>
<tr>
<td>DP6</td>
<td>7</td>
<td>31.535</td>
<td>0.0031</td>
<td>1.5753E-08</td>
<td>5.7501E-05</td>
<td>2.739E-05</td>
<td>High risk</td>
</tr>
</tbody>
</table>

Source: Data primer

Based on table 3 that the concentration of bacteria in Refill Drinking Water in the area around the Pattingaloang Health Center in Makassar City with 6 samples stated to have a high risk of disease.

Discussion

Microbial risk assessment is a process for calculating or predicting risk in a target organism, system or subpopulation, including identification of certainty that accompanies it, after being exposed by certain microorganisms by taking into account the inherent
characteristics of the cause (microbes) that are being studied and the specific target system characteristics\textsuperscript{15}.

Microbial risk assessment can be done in two ways based on data and the methods used both qualitatively and quantitatively. Risk assessment consists of four stages of study. The four stages are hazard identification, dose-response analysis (quantitative methods) and hazard characteristics (qualitative methods), exposure analysis and risk characteristics\textsuperscript{16}.

Based on table 3 it can be seen that the concentration for high risk levels, the concentration of germs that is for sample code DP1 colony / gr with a value of $P_{\text{inf.d}} = 6.967 \times 10^{-06}$ and $P_{\text{ill}} = \geq 1.63 \times 10^{-05}$, Code sample DP2 23 colonies / gr with $P_{\text{inf.d}} = \geq 5.23 \times 10^{-05}$ and $P_{\text{ill}} = 65.365 \times 10^{-05}$, Code sample DP3 1400 colonies / gr with $P_{\text{inf.d}} = \geq 3.61 \times 10^{-06}$ and $P_{\text{ill}} = \geq 3.26 \times 10^{-05}$, Code sample DP4 sample code 28 colonies / gr with $P_{\text{inf.d}} = 85.785 \times 10^{-05}$ and $P_{\text{ill}} = 61.761 \times 10^{-05}$, Sample code DP5 7 colonies / gr with $P_{\text{inf.d}} = \geq 1.57 \times 10^{-05}$ and $P_{\text{ill}} = \geq 2.73 \times 10^{-05}$, Column DP6 sample code / gr with $P_{\text{inf.d}} = \geq 1.57 \times 10^{-05}$ and $P_{\text{ill}} = \geq 2.73 \times 10^{-05}$.

From the results of a quantitative microbial risk assessment, it can be seen that E. Coli bacteria are very pathogenic to humans. Even with the concentration of safe risk being consumed continuously for a long time, it has provided a high risk for people who consume AMIU from some of the depots\textsuperscript{4}.

The results showed that there 3 depots whose water samples did not meet the requirements in terms of the MPN Coliform parameter because the coliform content exceeds 0 in 100ml sample samples water. Whereas for inspection based on Escherichia coli parameters, all samples were fulfilled requirements because the content of Escherichia coli in the water sample is 0 in 100ml sample sample water\textsuperscript{5}.

Identification of Escherichia Coli conventionally using biochemical reactions test and inoculation, it requiring quite a long time, the biochemical tests is hard to do, and are not accurate. This is because the bacterial colony alleged Escherichia coli in selective media and deferential media is often not pure and mixed with other Enterobacteriaceae bacteria\textsuperscript{17}.

According journal in makassar All depots that were sampled in Mariso Subdistrict did not handle the containers carried by the buyer in accordance with the regulation. The most common method used by most depots now is to brush and rinse with product water afterwards, then fill it immediately.In Mariso sub-district, 38.46% of the samples were brushing and rinsing and 60% of them produced drinking water with quality according to the regulations while the rest showed positive results. While the depot only rinsed, which was 46.15%, all the drinking water produced contained coliform bacteria. The rest of the depots who do not brushing and rinsing the container of the buyer are found to have total bac teriocoliform content\textsuperscript{18}.

Risk management is often used in the fields of financial investment, military planning and public health. In the field of public health, risk management is a policy making and risk control application that can endanger health (EPA, 2012). Microbial Risk Management (MRM) aims to control risk factors that are transmitted by pathogenic microbes that can cause health problems due to consuming water from polluted water sources. Therefore, policy making in microbial risk management is the responsibility of the government and NGOs working in the health sector and related communities so that the transmission of disease through microbes can be prevented or controlled.

One of the principles of Microbial Risk Management (MRM) is that risk management analysis and risk control strategies must be based on the level of risk (risk characterization). Risk characterization is a starting point for formulating risk management and providing a basis for decision making. In addition, risk management must also be based on a risk assessment\textsuperscript{10}.

According to the WHO Drinking Water Guidelines (2008), it suggests that the risk reference standard level is 10-6. So, if the value of $P_{\text{inf.d}} / P_{\text{ill}} > 10^{-6}$ (for example 10-5) then it is stated with high risk whereas if the value of $P_{\text{inf.d}} / P_{\text{ill}} < 10^{-6}$ (for example 10-7) then the risk is declared low. As for the value of $P_{\text{inf.d}} / P_{\text{ill}} = 10^{-6}$, then it is assumed to be of moderate risk.

In the Microbial Risk Assessment Guideline the steps described to make microbial risk management are: formulating problems in a broad context (planning and scoping), risk assessment, determining risk priority choices (risk characterization), making decisions that are appropriately, take action to implement decisions and conduct evaluations related to the effectiveness of the actions taken\textsuperscript{19}. 

Aspects of concern in risk management to overcome the occurrence of diseases, namely: Reducing the risk of disease caused by pathogenic germs by means that employees can pay attention to hygiene and sanitation starting from the processing, filling to sales in accordance with established regulations.

**Conclusion**

Based on a Quantitative Risk Assessment, it was concluded that of the 6 DAMIU samples having a high concentration of risk level, for the estimated amount of E. Coli bacteria in Refill Drinking Water in the area around the Pattingaloang Health Center in Makassar City.

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**Ethical Clearance:** Taken from by the Ethics Committee of University Moslem Indonesia

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Association of Early Nutritional Status with the Clinical Severity in Patients with Acute Stroke

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Abstract

Background: Prevalence of malnutrition in stroke patients in Indonesia is not known for certain because the assessment of nutritional status has not been conducted optimally in all stroke patients due to the existing limitations. Early nutritional status assessment is helpful in planning the provision of nutrition for acute stroke patients. Commonly used nutritional status assessment methods are anthropometry (LOLA and TST) and biochemistry (serum albumin levels).

Objective: To analyze the association of early nutritional status with clinical severity in acute stroke patients.

Methods: The study design was a prospective cohort with consecutive sampling according to inclusion and exclusion criteria of 102 subjects. Initial nutritional status was measured in Upper Arm Circumference (LOLA), Triceps Skinfold Thickness (TST), and serum albumin level. The clinical severity was measured by calculating the NIHSS (National Institutes of Health Stroke Scale) at admission to hospital and the 7th day of stroke. Data was analyzed using chi square (p <0.05).

Results: Most subjects were males (52.00%) with mean age of clinical severity was 54.48 ± 9.634 years old. There was no significant association between initial nutritional status (p = 0.227) and serum albumin level (0.552) with clinical severity. The measurements of LOLA (p = 0.049) and TST (p = 0.481) were statistically significant (p = 0.049) but not clinically.

Conclusion: There was no association between early nutritional status that was measured with LOLA, TST and serum albumin levels with clinical severity measured with NIHSS scale in acute stroke patients.

Keywords: early nutritional status, LOLA, TST, Albumin, acute stroke, NIHSS

Introduction

The incidence of stroke is 12.1 per 1000 population and stroke is considered as the highest cause of death in Indonesia. The high number of deaths due to stroke in Indonesia is influenced by many factors, one of which is the nutritional status of the patients. Malnutrition is often undetectable and an unresolved problem in stroke patients due to the low awareness and lack of education of hospital staff; thus, only a few malnourished patients receive adequate nutritional therapy.

The incidence of malnutrition in stroke in Indonesia is not known for certain because the assessment of nutritional status has not been a routine thing conducted in the management of stroke patients. The incidence of malnutrition in acute stroke patients ranged from 8.00 to 20.00% during the first day of treatment and increased to 35.00-50.00% in the first week of treatment with a prevalence of malnutrition in acute stroke as a whole was 6.00-62.00%. The difference in prevalence of malnutrition in stroke patients is quite wide because of the variety of nutritional status assessment methods used, the measurement time and the type of stroke.

Methods of frequent assessment of nutritional status are anthropometric and biochemical parameters. Examinations of upper arm muscle antropometry...
(LOLA) and triceps skinfold thickness (TST) are quite appropriate for bed rest patients and can roughly estimate lipid storage and muscle mass. TST values will decrease significantly in acute stroke patients on day one and 7 days after treatment. Serum albumin levels are frequently used biochemical nutritional assessments. Albumin is mentioned to have a strong value in diagnosing malnutrition in stroke. Serum albumin concentration will decrease during the first day and one week after treatment in acute stroke patients.

Stroke causes a negative energy balance and increases nutritional needs, but few stroke patients can fulfill the increased need. Malnutrition is one of the poor clinical outcome indicators months post stroke and low albumin levels on the first day of treatment to be an independent predictor of poor clinical outcomes. Malnutrition can lead to high mortality in the long term. Early nutritional status assessments can greatly assist nutrition planning for patients, and given the high prevalence of malnutrition in patients with acute stroke and the magnitude of the impact, the researchers intended to examine the association of initial nutritional status assessed with TST, LOLA and serum albumin levels with clinical severity assessed with NIHSS in acute stroke patients.

Method and Materials

The subjects of the study were acute stroke patients treated in Dr. Soetomo General Hospital Surabaya from April to August 2017. The inclusion criteria were acute stroke, either infarct stroke or bleeding stroke diagnosed clinically with Computed Tomography scans (CT-scan) head without contrast, age >18 years old, and first onset stroke <48 hours. Exclusion criteria included patients with malignant tumors, symptoms and signs of sepsis when admitted to the hospital, suffering from acute and chronic kidney failure, and impaired hepatic function.

The study design was a prospective cohort. The sample collection was conducted by consecutive sampling admission technique. The major subjects of the study were determined by the sample formula for unpaired categorical analytic research, two-way hypothesis with type I error of 5.00% and type II error of 20.00%. The subjects of the study were divided into two groups, namely the acute stroke subject group with malnutrition and the stroke subject group with good nutrition. At the end of the research, we obtained a large sample of 102 subjects. Prior to the identification of the subject first, the researchers conducted a test of ethics (324/Panke.KKE/V/2017) in Dr. Soetomo General Hospital Surabaya, Indonesia.

Anamnesis was performed to collect demographic data (age and gender) as well as clinical data (type of stroke, blood pressure, random sugar levels, and smoking status). The subjects were examined for initial nutritional status measured by TST, LOLA and serum albumin levels. A good nutritional status was shown if all TST, LOLA and serum albumin levels were normal and poor nutritional status if at least one of the measurements of TST, LOLA and serum albumin levels was below normal. TST was measured with caliper skinfold. Normal TST value of male patients was 12.5 mm and female patients was 16.5 mm. LOLA was measured by a tape measure of the formula LOLA = [0.1 LLA (mm) - (0.314xTST (mm)] . The normal value of LOLA in males was 23 cm and female was 20 cm. Levels of serum albumin were examined from the blood of the subjects within <48 hours of stroke treatment. Normal albumin level was 3.5 g/dL. The clinical severity was measured by using NIHSS scale. Measurement of clinical severity with NIHSS scale was performed on the first day of admission and 7th day of onset. The data was analyzed using chi square statistic test with SPSS version. 20.0 (SPSS, Inc., Chicago, IL).

Results

Basic Data Characteristics of Research Subjects

The majority of research subjects were male patients (52.00%). In the group with clinical severity, the research subjects with male gender (69.80%) were greater than female subjects (57.10%) (table 1). The mean age of the study subjects in the group with a fixed clinical severity was 54.48 ± 9.634 years and the mean age of the study subjects in the group with improved clinical severity was 59.05 ± 11.935 years (table 2). The mean gender difference (p = 0.84) and age (p = 0.331) in both groups was not statistically significant.

Clinical Data of Research Subjects

The clinical severity in the group of subjects with bleeding stroke (64.70%) was greater than stroke infarction (63.20%). Subject groups with normotension (70.00%) were greater than hypertension (63.00%) at a constant clinical severity. The clinical severity in the subjects group with hyperglycemia (64.03%) was greater than normoglycemia (63.30%). Group of non-
smoking subjects (63.80%) was greater than smoking (63.60%) in the degree of permanent clinical severity. There was no significant difference between stroke (p = 0.884), hypertension (p = 0.744), hyperglycemia (p = 0.922) and smoking (p = 0.990) with clinical severity (table 1).

Association of Early Nutritional Status with the Clinical Severity

The degree of clinical severity subjects with malnutrition status (68.30%) was greater than in subjects with good nutritional status (56.40%) and it was not statistically significant (p = 0.227). In LOLA measurements, the clinical severity in the group of subjects with malnutrition status (73.50%) was greater than good nutritional status (54.70%).

In TST measurement, the clinical severity in the group of subjects with good nutritional status (65.40%) was greater than malnutrition status (57.10%). In measurement of serum albumin level, the clinical severity in the subjects group with malnutrition status (100%) was greater than good nutrition status (62.6%). The measurement of the fixed clinical severity towards nutritional status with LOLA (p = 0.049) and TST (p = 0.481) had statistically significant differences compared with serum albumin levels (p = 0.552). However, the measurements of LOLA (RR = 1.343, 95% CI 1.999-1.997) and TST (RR = 1.597; 95% CI 1.371-1.859) were also not clinically significant (table 3).

Discussion

The majority of research subjects were males although the percentage was not much different from female subjects. Males have a higher risk of having a stroke than females, especially in the <65 years age group. In this study, the mean age of study subjects in the group with fixed clinical severity was 54.48 ± 9.634 years and mean age in the group of subjects in the improved clinical severity group was 59.05 ± 11.935. This result was not much different from epidemiological data from 28 hospitals in Indonesia which stated that the mean age of stroke patients was 58.8 ± 13.3 years.

Confounding variables such as hypertension, stroke type, hyperglycemia and smoking do not show statistically significant proportions. Thus, these confounding factors can be neglected although in other studies stated that confounding variables have an influence with the severity of clinical stroke. There was no significant association between initial nutritional status and clinical severity. Inappropriateness with the research hypothesis may be due to the observation period for assessing clinical severity is only the first 5-7 days of acute onset of stroke. Research in China showed a poor clinical outcome (MRS scale) at 3 months post stroke. Extended observation may increase the number of clinical significance but can increase the number of drop outs for patients who undergo outpatient care.

Second, this study measured only two anthropometric parameters and one biochemical parameter as a parameter of nutritional status of acute stroke patients. The more examination of nutritional status, the value of the measurement of nutritional status is stronger, because until now there has not been one method used as a gold standard in the assessment of nutritional status.

Third, nutritional status is presumed to be only a minor risk factor in affecting the degree of clinical severity in stroke patients. Poor clinical outcomes in stroke patients are caused by age at the onset of stroke, severity at the time of stroke, disability suffered before stroke, comorbid factors and complications arising from infection.

At LOLA and TST examinations, there was a statistically significant difference in proportion but not clinically significant. The results of the analysis on both anthropometric parameters can be caused by the cut off used. This study used a cut off of LOLA and Dutch TST (Caucasian race). In Indonesia, research on cutoffs for LOLA and TST examinations on populations is still limited. Assessments of dietary intake, mobility or activity performed before illness or other factors are required to be taken that may affect the outcome of the nutritional status parameters used.

Most subjects had good nutritional status from the point of biochemical parameters. This can be seen from the low subjects with hypoalbuminemia. Hypoalbuminemia is a predictor of poor clinical outcomes as measured by MRS scales three months after stroke onset. In acute ischemic stroke is associated with mortality in the first 7 days.

Although there are studies that examine the relationship between nutritional status and hospitalization and geriatrics, there has been no research in Indonesia that specializes in nutritional status with clinical severity in acute stroke patients. This study is the first study to find the relationship between initial nutritional status.
with clinical severity in acute stroke patients. In addition, this study used a prospective cohort design study which is the best observational research design to find out the cause of a case.

**Conclusion**

The majority of subjects were male patients with the average age of more than 50 years old. The majority of subjects had a type of infarct stroke, high blood pressure, normoglycemia, and no smoking. Initial nutritional status as measured by TST, LOLA and serum albumin levels did not correlate with clinical severity measured using NIHSS scales in acute stroke patients. Factors that influenced can be a short observation time, cut off value used, the limited tool during the examination of nutritional status, and nutritional status is a minor factor that affects the clinical severity in acute stroke patients. Further research is required by measuring the degree of clinical severity over a longer period of time, increasing the parameters of nutritional status used and assessing changes in nutritional status before and after stroke treatment in relation to clinical severity of acute stroke.

**Conflict of Interest:** There is no conflict of interest.

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The Legal Protection of Well-Known Service Trademark (The Case of Inter-Continental Vs The Intercontinental)

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Abstract

The competition in the field of international services has increased the potential for copyright infringement of well-known service marks. Accordingly, it is necessary to examine the criteria for well-known service marks and legal protection to protect the trademarks’ copyright. This paper analyzes the criteria for well-known service marks according to the instruments of international law and national law. Besides, this study aims to investigate the forms of well-known service mark violations and the efforts to recover the trademarks. The method employed in this study was normative legal research. That method was used since this thesis examined the norms in the laws and regulations as well as the judgments relating to the trademarks to find the law against the issue addressed by utilizing statute approach and conceptual approach. In the case of INTER-CONTINENTAL v. the intercontinental, the trademark violation occurred was brand dilution. The parties in the INTER-CONTINENTAL v. Intercontinental case could resolve their dispute through alternative dispute resolution in the form of mediation, conciliation, or arbitration. In addition, the act of trademarks counterfeiting and piracy may be subjected to criminal sanctions according to the provisions of the applicable Trademark Law. The infringement of trademark copyrights is a detrimental act for the trademark’s legal owner. The settlement of trademark infringement can be conducted through criminal channels and alternative dispute resolution by mediation, conciliation, or arbitration.

Keywords: well-known service mark, criteria, forms of violation, and recovery efforts.

Introduction

The development of global trade causes an urgency for international brand protection. 1, 2. Nowadays, the brand forms a business strategy tool in order to win a very competitive competition. 3. The use of well-known service marks in trade has the potential to ease someone with bad intentions to market their products. It is caused by the fact that well-known brands have gained recognition and trust from consumers since they were known to have high-quality standards. To gain profits quickly, a person can imitate, profiteer, or even counterfeit the famous brands, which certainly causes harm to legitimate brand owners, consumers, and even the State 4. One of the notorious cases is the inter-continental brand plagiarism, which is the legally valid property of the INTER-CONTINENTALS HOTELS CORPORATION based in the United States. INTER-CONTINENTALS HOTELS CORPORATION as a plaintiff sued for violations committed by PT. Lippo Karawaci as a defendant for using the intercontinental trademark without the owner’s permission, as well as the Government of Indonesia, in this case, the Ministry of Law and Human Rights and the Directorate General of Intellectual Property Rights (Directorate of Trademarks) 5. This study discusses two important issues, i.e., identifying the criteria of famous service marks and analyzing the violations of famous service marks along with the recovery efforts.

Methods

The method employed in this study was normative legal research. That method was used since this thesis examined the norms in the laws and regulations as well as the judgments relating to the trademarks to find the law against the issue addressed by utilizing statute approach and conceptual approach.
Results and Discussion

Famous Service Mark Criteria According to International Law Instruments

TRIPs (Agreement on Trade-Related Aspects of Intellectual Property Right) is a more recent regulation related to brand protection. To implement the TRIPs agreement and concurrently develop national laws on IPR, Indonesia is currently preparing the regulations on IPR. The trademark law has changed several times since it was first enacted in 2001. At present, the applicable law is Law No. 20 of 2016 concerning Marks and Geographical Indications, namely the 2016 Trademark Law. TRIPs contain three main issues. First, it contains general rules and basic principles as guidance to the WTO member countries. Second, it contains the standards regarding the administration, use, and scope of each IPR mentioned in TRIPs. Third, it contains provisions relating to the WTO member countries’ obligations to carry out law enforcement on IPR and legal remedies that can be taken to protect and maintain IPR (Ramli, 2000). TRIPs as an international agreement has relevance to agreements and other international conventions on IPR (Usman, 2015).

According to Article 16 paragraph (1) of the TRIPs, the Brand Owner is obliged to register his mark in the territory of the participating country if he wants to acquire exclusive rights. Exclusive rights give rise to monopoly rights over a trademark. Thus, it can be stated that the existence of these rights emerges a legal monopoly. Trademark adheres to the territorial principle, which implies that brand protection only applies in the country where the application for the mark is submitted and granted. To obtain brand protection in the Indonesian jurisdiction, the trademark owner must submit a trademark application in Indonesia. The regulation on the famous mark stated in the TRIPs Agreement is a continuation of the regulation from the Paris Convention. The INTER-CONTINENTAL brand is a service brand that is engaged in hospitality owned by multinational companies and is registered in more than one hundred countries in the world. Legally, the INTER-CONTINENTAL brand should have fulfilled the criteria of a famous mark. Due to INTER-CONTINENTAL’s large investments in various countries in the hospitality sector since 1949, the brand should have been known by the public at large in various countries, especially in the relevant field of hospitality services.

Famous Mark Criteria According to National Legal Instruments

The registration of Intercontinental Trademark owned by the Defendant should be denied by the reasons contained in article 21 paragraph (1) of the 2016 Trademark Law because the intercontinental trademark has similarities in basics or on the whole with the Plaintiff’s trademark, i.e., the INTER-CONTINENTAL. Hence, it can give the impression that the Defendant and the Plaintiff have a relationship, attributed to the similarity. According to Rahmi Jened in Buku Hukum Merek Dalam Era Global dan Integrasi Ekonomi, the reasons for rejection in article 21 paragraph (1) of the 2016 Trademark Law are the relative grounds. Therefore, the Defendant’s trademark rejection reasons should be based on relative grounds.

The Plaintiff’s trademark has been registered at one hundred countries in the world. The Plaintiff’s trademark has been registered in one hundred countries in the world. INTERCONTINENTAL Plaintiff’s Trademark should have fulfilled the criteria of a famous mark. According to Law No. 15/2001 or 2016 Trademark Law, the definitions of famous mark are not clearly stated, but in the Elucidation chapter of Article 21 Paragraph (1) Letter b of the 2016 Trademark Law, famous mark must be considered or can be marked with a. the basis of public knowledge about the brand; b. the brand’s reputation is obtained through intensive and extensive promotion; c. trademark registration is carried out in several countries and d. the company’s investment in other countries.

Concerning the INTER-CONTINENTAL against PT. LIPPO Karawaci case, the Plaintiff’s Trademark, INTER-CONTINENTAL, has been registered in one hundred (100) countries in the world, including Indonesia, and historically the Plaintiff’s famous service mark has existed and been traded since 1949. The Plaintiff proved it with the evidence basis, i.e., a copy of the registration mark certificate from twenty-nine (29) countries, including Indonesia. In business, to create markets in various parts of the world requires capital, a very large amount of investment, and a long period. Thus, a famous mark must be protected. In this case, it is necessary to prove whether the Plaintiff’s trademark is a well-known mark or a famous mark. Famous mark is considered a higher reputation than a well-known mark since it is in a superlative form and it is the highest level of the famous brand based on the word meaning. Famous mark requires at least registration of a mark in
its own origin country.

**The Forms of Violations and Recovery Efforts for Trademark Violations**

Famous brands are prone to trademark violations, such as counterfeiting and piracy. In principle, trademark violations are based on bad faith from the violation perpetrators. Article 41 of the TRIPs states that: “Members shall ensure that enforcement procedures as specified in this Part are available under their laws so as to permit effective action against any act of infringement of intellectual property rights covered by this Agreement, including expeditious remedies to prevent infringements and remedies which constitute a deterrent to further infringements. Based on the above provisions, TRIPs requires that there is a rapid recovery effort for a trademark violation as well as a good preventive effort. Strictly stated in Article 41, TRIPs mentions the examples of violations in general, which is Trademarks Infringements.

Furthermore, brand infringement can be in the form of a Passing Off, which occurs when someone sells a product as if it were a product of the famous brand and has a good reputation, or at least allude a relationship that confuses the community, which those gives a loss to the real brand owner. The Second is Dilution. Dilution is a weakening or reducing the ability of a famous brand to distinguish goods and services without paying attention to any confounding similarities, so the consumer will not be confused in distinguishing a product even though the brand name is the same. Therefore, the distinguishing and unique characteristics of the brand are reduced by the brand similarity and likeness in principle. Dilution damages the reputation of famous brands through these actions. The Third is Counterfeiting and Brand Piracy. Counterfeiting and brand piracy can be detrimental to many parties ranging from the community, brand owners, and also the state which loses the revenue from the sales tax sector while the pirated goods are sold without tax. The consumers as users will be aggrieved due to those actions. (Agung Sujatmiko a, 2008).

**The Recovery Efforts for Famous Trademark Violations**

Concerning the INTER-CONTINENTAL against PT. LIPPO Karawaci case, the violation occurred was brand dilution. Even though dilution does not confuse because it only weakens the brand, but it is a violation of the famous brand owner’s exclusive rights. Therefore, the famous brand owner suffers a loss. Furthermore, in the Elucidation Chapter Article 76 of the 2016 Trademark Law, it is stated that the parties that can file a trademark cancellation claim are: Concerned parties are parties who legally have personal interests that are legally recognized. Anyone with interest in intellectual property, such as the right to the brand and the right to use the brand, prosecutors, foundations/agencies, religious assemblies/institutions. The trademark owner who is unregistered after submitting an application for trademark registration in good faith to the Directorate General of Intellectual Property or the owner of a well-known mark yet it is unregistered.

After taking legal action against the cancellation, the Plaintiff’s claim was rejected by the judex factie judicial panel of the Central Jakarta Commercial Court. In its decision, the judex factie assembly rejected the Plaintiff’s claim in its entirety. In its legal considerations, the judex factie panel believed that the Defendant and Defendant’s trademarks were dissimilar because of the capital letters used and writing differences. Hence, the similarity element was essentially unproven, so the Defendant’s trademark rights were legal. According to the article 78 provisions of the 2016 Trademark Law, to the Commercial Court decision, only the appeal may be submitted. Therefore, the Plaintiff filed an appeal to the Supreme Court. The Plaintiff’s appeal request was granted partially by the Supreme Court which stated emphatically that the Plaintiff’s trademark was classified as a famous mark category, so it was also protected for non-similar goods. The judex juris of Panel of Judges canceled the judex factie decision and stated that the Defendant’s trademark in the form of speech or voice said essentially resembled the Defendant’s trademark, so it had to be canceled.

Based on the provisions of article 85 of the 2016 Trademark Law, a civil action is filed with the Commercial Court, where the Defendant’s law or the offender domiciles (actor sequitur forum rei). However, if the Defendant is abroad, the lawsuit is filed in the Central Jakarta Commercial court. The provisions regarding the right to file a lawsuit by the owner and/or holders of license over mark are regulated in Article 83 of the 2016 Trademark Law that the registered Trademark Owner and/or Holders of License Over Mark can file a lawsuit for violation against another party without the right to use a Trademark that has similarities in basics or on a whole for similar goods or services in the form of
IPR criminal provisions on trademarks are in Article 61 of TRIPs. The violation of a famous service mark may be subjected to criminal sanctions if the violation is carried out by brand counterfeiting or brand piracy. Brand counterfeiting here is undertaken by faking existing brands, which can create an impression on the public as if the counterfeited goods are the same as the original goods that are using famous brands to related institutions 19. Article 100 of paragraph (1) of the 2016 Trademark Law states that any person who without the right to use the Mark as a whole in its entirety with another party’s registered Marks for similar goods and/or services that are produced and/or traded, is convicted with a maximum criminal imprisonment of 5 (five) years and/or a maximum fine with no more than Rp. 2,000,000,000.00 (two billion rupiah). This provision provides strict sanctions for the perpetrators of counterfeiting brands. Furthermore, in Article 100 of Paragraph (2) of the 2016 Trademark Law states that “Every Person who without the right to use the Mark which has the similarities in principle with a registered Mark that belongs to another party for similar goods and/or services that are produced and/or traded, is convicted to a maximum criminal imprisonment of 4 (four) years and/or a maximum fine of Rp. 2,000,000,000.00 (two billion rupiah). “ This provision provides strict sanctions for perpetrators of brand piracy. According to Rahmi Jened 72, the terminology of brand piracy in TRIPs is translated as similarity in principle in the 2016 Trademark Law, so that anyone who commits brand piracy may be subject to criminal sanctions according to the provisions of article 100 of paragraph (1) of the 2016 Trademark Law. Article 93 of the 2016 Trademark Law which regulates that in addition to taking civil actions, the parties can also settle the disputes in article 83 of the 2016 Trademark Law through Arbitration or Alternative Dispute Resolution. Based on these provisions, then in the case of INTER-CONTINENTAL v. the intercontinental, can be resolved through alternative dispute resolution (amicable settlement), where the parties voluntarily agree on a collective agreement (party autonomy principle) to settle the disputes outside the court. According to the elucidation Chapter of Article 93 of the 2016 Trademark Law, the forms of alternative dispute resolution include negotiation, mediation, conciliation, as well as other means chosen by the parties 11,20 but the regulation of domain name crimes is not regulated in the ITE Law as mandated in the academic draft of the ITE Bill. The absence of regulation of domain name norm in the ITE Law creates problems with registrant of domain name (registrant).

Conclusion

In the case of INTER-CONTINENTAL v. the intercontinental, the trademark violations occurred are brand dilution. The parties in the INTER-CONTINENTAL v. Intercontinental case can resolve their dispute through alternative dispute resolution in the form of mediation or arbitration. In addition, counterfeiting and trademark piracy may be subject to criminal sanctions according to the provisions of the applicable Trademark Law.

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Determining the Quality of Services Provided in Delivery Room at Ayatollah Kashani Hospital (Jiroft, Iran) from the Perspective of Clients Using Service Quality Model (SQM) During Spring 2019

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Abstract

Background and Objective: Topnotch services provided through preserving high quality is a prerequisite to the success of service organizations including maternity hospitals. Since maternity hospitals play an important role in providing services and promoting women’s health and that of the entire society, this study attempted to assess the quality of services provided in the maternity ward of Ayatollah Kashani Hospital in Jiroft (Iran).

Method: This was a cross-sectional, descriptive study conducted on 234 women who went through vaginal delivery in Ayatollah Kashani Hospital. After 2 hours of delivery, convenience sampling was carried on the subjects in 2019. Data were collected through SERVQUAL standard questionnaire (1988), the validity and reliability of which were confirmed. Data were analyzed by SPSS using descriptive and inferential statistics.

Results: The results indicated that there is a negative gap in all dimensions of service quality. The largest gap was observed in assurance (-5.69) while the lowest gap was in the physical dimension (-3.2). There was a significant difference between women’s expectations and perceptions across all five SERVQUAL dimensions (P<0.001).

Conclusions: From the women’s perspective, the quality of services was not satisfactory and the significant difference between expected and received services indicates that managers should pay more attention to quality improvement programs in maternity hospitals so that increasing the quality of services will promote women’s health and eventually that of the entire society.

Keywords: Women, Maternity Hospital, SERVQUAL, Quality of Health Care

Introduction

Nowadays, promoting the society’s health is a key factor in economic and social development. Therefore, one of the major concerns of countries worldwide is assurance of health care quality, programs and services. Quality is a main factor in global competition, where managers have to provide quality services for successful competition (1-2-3). Quality of service is the comparison of what the client feels it should be (expectations) with what it has actually received (perceptions). If expectations are higher than perceptions, the client would perceive the quality of service to be lower, leading to dissatisfaction (4). Expectation is one of the most important determinants of client evaluation of service quality, and accurate understanding of client expectations is the most important step in defining and delivering high-quality services. In fact, one of the current challenges in the health system is how to respond to patient expectations. This has been neglected despite the importance of recognizing the needs and requirements of patients in providing care (5). The

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results of various studies demonstrate that fulfilling patient expectations is associated with high satisfaction with services, whereas unfulfilled expectations are associated with dissatisfaction. Patient satisfaction is one of the critical goals of group therapy activities. Patients’ satisfaction with hospital services is one of the most important indicators of quality and effectiveness in service delivery and has made a major contribution in this regard. Satisfied patient is a key factor in any hospital’s success. In fact, patient satisfaction is defined as patients’ overall perception of the quality of healthcare provided (6-7). The only way for the patient to participate in caregiving is to research and seek patient satisfaction with current structures and to obtain their feedback. One of the most important groups under study in the field of satisfaction is patients in care and hospitalized in the maternity ward. That is because the delivery process during the care period is extremely stressful and it is one of the undesirable experiences of many people (8-9). According to a statement by the International Confederation of Midwives (ICM) and the International Federation of Gynecology and Obstetrics (FIGO), awareness of the disadvantages and benefits of different therapies, participation in the decision-making process and offering high quality services are among the ethical rights reserved for pregnant women (10). Observance of these rights leads to greater satisfaction with prenatal care and increased satisfaction with childbirth and, consequently, lower rate of cesarean delivery and the complications of pregnancy and childbirth on the mother and fetus (11). Accordingly, mothers can participate in their own treatment process and receive more quality therapeutic interventions (12). Deploying high-quality services saves costs, enhances the effectiveness of specialized service providers, strengthens staff morale, creates a satisfying environment, and increases patient loyalty in choosing a hospital for health care.

Conflicting perceptions of service quality have led to the adoption of different methods to assess service quality (13). For many years, researchers have been measuring quality of service using one-dimensional scales, while one-dimensional scales are not suitable for measuring a multi-dimensional concept such as quality (14). One of the best and most practical strategies to evaluate service quality involves SERVQUAL, a model designed by Parasuraman et al. (2008). SERVQUAL is a method for purposefully identifying the strengths and weaknesses of service organizations such as hospitals. It is also used to comparatively measure the perceptions and expectations of clients (patients). The SERVQUAL scale contains metrics, half of which measure the expectation level of service recipient while the second half measures the perceived level of quality provided by the organization (15). Given that patient satisfaction assessment can provide the basis for identifying the current strengths and weaknesses and pondering strategies for providing better care and improving the quality of services, SERVQUAL was adopted as a powerful tool for measuring service quality (16-17-18). Moreover, given that quality analysis of hospital services enables hospital managers to allocate funds to improve performance in areas that have a greater impact on patient satisfaction, and since our preliminary study about quality of service in Iran was more focused on primary health care without using expert opinions, we intended to conduct a research to evaluate the quality of services provided in Ayatollah Kashani Hospital in Jiroft during spring 2019. This was decided because fewer authorities have taken this into consideration despite the importance of maternity ward’s emergency department in ensuring the health of pregnant mother and her fetus, as well as the special importance of Ayatollah Kashani Hospital Maternity Hospital as the only specialized women’s hospital in southern Kerman.

The most important client will be realized through proper planning, correction of deficiencies and ultimately improvement of service quality (26).

Materials and Method

This is a cross-sectional, descriptive-analytical research project. The statistical population includes all women referred to Ayatollah Kashani Hospital in Jiroft for the period of March to June 2019. The data were collected by census method and from all qualified clients. Inclusion criteria: Informed consent to participate in the study, maximum 5th pregnancy, at least 24 hours of hospitalization, pregnant or 2 hours past parturition and transferred to gynecology ward, maximum age 45 years old, minimum literacy reading and writing, no physical or mental illness. Moreover, women in the active phase of labor who did not complete all sections of the questionnaire were excluded. Finally, the population comprised 234 subjects. After obtaining permission from the Ethics Committee and agreeing to hospital managers, the leading researcher attended the maternity ward and administered the SERVQUAL questionnaire at different labor shifts (morning, afternoon, and evening) until the end of June. The subjects returned their
written consent and were assured of the confidentiality of their information. The questionnaire consisted of two parts: demographic information and SERVQUAL items. Personal information involved 11 items (age, number of pregnancies, level of education, etc.) The second part covers SERVQUAL Multidimensional Questionnaire. The questionnaire included 28 items in 6 dimensions, namely physical (5 items), reliability (5 items), responsiveness (5 items), service assurance (5 items), empathy (4 items), and access to care (4 items), while the questionnaire was based on a five-point Likert scale: very high, high, medium, low, very low. In their study, Mousavi et al. calculated reliability and validity of the questionnaire through Cronbach’s alpha, which were 0.88, 0.87, 0.88, 88. 0, 0.91 and 0.86 in physical dimension, responsiveness, assurance, empathy, reliability and accessibility, respectively (28). In order to enhance the concentration of subjects, the questionnaire was administered without staff presence. It took about 15 minutes to complete the questionnaires. After collecting the questionnaires, data were analyzed using SPSS 20. Furthermore, the data normality assumption was evaluated by Kolmogorov-Smirnov test and, Mann-Whitney and Kruskal-Wallis nonparametric tests were used.

Results

A total of 234 subjects with a mean age of 27.0±6.44 participated in the study, of whom 80% held a diploma degree and lower, while the rest had a bachelor’s degree or higher. Of these, 87% were housewives and about 9% were occupied, 39% experienced first pregnancies and 5% were on the fifth pregnancy. There was no significant relationship between age and satisfaction (p=0.56). The mean gap score in patients with a bachelor’s degree and higher was significantly higher than those with a lower level of education (p<0.05). Interestingly, mean expectations were similar in the two groups (p=0.08) but mean perceptions in the bachelor and higher groups were significantly lower (p<0.05). The mean score of gap was significantly higher in those with a bachelor’s degree and higher (p<0.05). The mean gap score was significantly different between those who tended to be pregnant and those who did not tend to be pregnant (p=0.39).

As can be seen in the table below, physical dimension had the smallest gap (-3.2) while assurance indicated the highest gap (-5.69). Individuals expressed the highest expectations in terms of accountability and assurance while the lowest perceptions were in empathy and access. There was a significant difference (p<0.001) between mean perceptions and expectations at all dimensions.

Discussion

The main purpose of this study was to investigate the level of expectations and perceptions about the quality of services from the perspective of patients referred to Kashani Hospital in Jiroft during spring.

Similar to many researchers, we attempted to examine the gap between client expectations and perceptions of service quality. According to the findings in our study, the lowest and mean gaps and highest perceptions were found in the physical dimension which was inconsistent with the findings of Seyedi et al. (29). This reflected that the hospital cared for physical and infrastructural facilities similar to the results of a periodic survey conducted between 2000 and 2008 focusing on the provision of services at a dental service facility in Estonia. Their research results showed that the gap between patients’ perceptions and expectations of service quality widened between 2000 and 2007 and decreased during 2008 (30).

Furthermore, the low score of perception and expectation in the empathy dimension indicates that the hospital-patient interactions have weaknesses that need to be addressed. This is in contrast to the results of Gray Boshoff (31) and Sidey et al. (29). It is also noteworthy that the individual interactions that occur during the care processes have a significant impact on the recipient’s perception of the quality of service provided by the medical center.

The highest expectations and gaps were found in assurance, which is consistent with the results of the study by Mousavi et al. (32) as well as Baker (33). These results indicate that from the patients’ perspective, the proficiency and expertise of the medical staff and the protection of patient rights when providing care services are vital, and the large gap in this dimension indicates that their expectations and dissatisfaction are not met.

In this study, the mean score of expectations in patients with university and non-university education was the same, but there was a significant difference in the perception of these two groups, which resulted in a significant gap in education, so that the mean of the academic gap was higher than the others. Greater level of education increases the level of expectation and
consequently decreases the perception of quality.

In our study, the variables history of hospitalization, insurance coverage and length of hospital stay did not lead to any significant difference in the main variables.

**Conclusion**

Finally, we suggest that the gap across all items is significant, which means that the hospital has a long way to go before satisfying the patients in providing health services.

**Conflict of Interest:** We declare that there is no conflict of interest.

**Source of Funding:** None

**Ethical Clearance:** Taken from Jiroft University of medical sciences.

**References**


2005.


Correlation between Serum & Urinary Placental Protein (Pp13) in Pre-eclamptic Women at their Third Trimester

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Abstract

Background: Preeclampsia (PE) consider as one of the most serious complications of pregnancy, and the chief reasons of

Objectives: Study the correlation between serum & urinary placental protein (pp13) in pre-eclamptic women at their third trimester

Materials and Method: Case control study was achieved from August 2018 to January 2019. A total of 100 women involved in this research, 50 preeclampsia pregnant women and 50 apparently normal pregnant women as control, for all the subjects in the research serum and urine sample were collected for placental protein 13 (pp13) estimation by using ELISA technique.

Results: Results indicated that a highly significant decreased in serum and urine pp13 in preeclamptic women compared to healthy women pregnant

Conclusion: Maternal serum PP13 at their 3rd. Trimester has evidences to be a credible biomarker for preeclampsia risk evaluation: the specificity and sensitivity of pp13 provided the highest diagnostic for preeclampsia

Keywords: third trimester, preeclampsia, PP13.

Introduction

Preeclampsias (PE) consider as one of main pregnancy disorders that has worldwide incidence rates about 5–8% [1]. The delivery of the placenta is the only acknowledged cure. PE is the major reason of the premature birth in developed countries, generally physically shown for the assistance of the mother [2].

PE is capable of being separated into two major kinds, the early and the late onset PE. This classification is depending either on the onset period or the identification of the syndrome [3]. The late onset PE comprises the majority (>80%) of preeclamptic. In the early onset type, the clinical symptoms appear prior to 33 weeks of gestational, while regarding late onset kind they happen following 34 weeks. The early-onset type that is accountable for most of the high maternal and fetal mortality and morbidity rates [1].

The American College of Obstetrics and Gynecology defined the clinical diagnosis of PE which involves blood pressures more than 140/90 mm Hg on 2 occasions joined with excretion of protein in urine more than 300 mg/d. The word Edema, considered as a classic characteristic of this disorder, it is not yet regarded as a detection sign due to absence of validity indicators the eclampsia could be existing in 20 percent of patients without previous proteinuria or hypertension, suggestive of the presently using biomarkers for diagnosis are not ideal [4].

Placental protein 13 (PP13) is a 32 kDa dimer protein which is produced just in the placenta also it is believed to be included in maternal artery remodeling and usual placentation. It was found by Nicolaides et al. [5] that a remarkable decrease of serum PP13 values at 11 to 13
gestational weeks in the women whom consequently resulted the early type of preeclampsia, which was then validated by Spencer et al. [6].

Galectins (GAL) are many purposely controllers of primary cellular methods. They are as well included in natural and adaptive immune responsiveness and achievement aoperative role in the immune endocrine crosstalk. A number of GAL have invited interest in the reproductive knowledge for the reason that they are very much expressed at the maternal fetal line, their purposeful importance in eutherian pregnancies, and their unregulated illustration is detected in the “huge obstetrical disorders.” Those GAL; could function as significant proteins included in maternal fetal connections. The examiner of these GAL can progress the predictive, detection, and therapy of the pregnancy women difficulties [7].

Methods

A case control study was carried out on 100 pregnant women over a period of seven months from August 2018 till January 2019. The participants in this research were collected from different hospitals in Baghdad city; the Al-Elweyia, Al-Hakeem, and Al- Imamain alkadhimain medical city. The practical fraction was accomplished at Research Laboratories in the Department of Chemistry and Biochemistry, College of Medicine/Al Nahairn University.

All subjects who included in the research were subjected by their physician to physical examination, blood pressure measurement, and laboratory investigations including serum and urinary pp13.

Inclusion criteria: All pregnant women included in this study were chronic hypertension pregnant women at their third trimester, gestational hypertension, renal and liver diseases, diabetes mellitus, smokers, fetal structural anomalies, multiple pregnancy, intrauterine fetal growth restriction from other causes, heart failure, inflammatory disorders, elderly pregnant, infectious disease, endocrine disease, HELLP syndrome and collagen vascular disorder were all expelled from the study.

Five milliliters (ml) venous blood had been withdrawn from all pregnant women by the use of disposable syringes in the sitting situation. Then it was discharged gradually in disposable test tubes without anticoagulant. And waited for clotting at 37°C for 10 to15 minutes, then centrifugation at 1000 xg for about 10-15 minutes. Their serum was stored eppendorf tubes at -80°C until analysis of pp13.

Random urine sample was collected in asterilelecup for urine collection. Pregnant women who participate were informed to throw away the initial20-25ml of urine and have a collection ofapproximately60 mL urine of mid stream and stored in at -80°C until analysis of placental protein 13(pp13).

Results

The mean ± SE of maternal age for control group and PE groups (mild and severe cases) were 29.28±1.08 years, 29.84±1.70years, 29.85±1.27 respectively. No significant difference (P= 0.931) was found between them.

<table>
<thead>
<tr>
<th>Type</th>
<th>Control</th>
<th>Mild</th>
<th>Sever</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Mean±SE</td>
<td>Mean±SE</td>
<td>Mean±SE</td>
<td>0.931NS</td>
</tr>
<tr>
<td></td>
<td>29.28±1.08</td>
<td>29.84±1.70</td>
<td>29.85±1.27</td>
<td></td>
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</tbody>
</table>

The mean ± standard error of mean of serum pp13 for control group and PE groups (mild and severe cases) at 3rd trimester afterbirth were 67.06± 3.296 pg/ml, 54.29± 3.129 pg/ml, 54.33 ±4.312 pg/ml, 54.25 ±4.64 pg/ml respectively.

There was highly significant increased (P=0.006) in mean of serum pp13 between control versus patient group

Also, high significant decreased (p=0.026) was found between mild preeclamptic group versus control group, while a highly significant decreased (P=0.035) was found between severe preeclamptic group versus control pregnant (Table1-2), but no significant difference (P=0.990) was found between mild against sever group.

The mean ± standard error of mean of urine pp13 for control group and PE groups mild and severe cases
at 3rd trimester after birth were 51.84±2.601 pg/ml, 38.39±2.717 pg/ml 43.44± 4.914 pg/ml, 33.34 ±1.863 pg/ml, respectively.

There was a highly significant decreased (P=0.001) in mean of serum pp13 between patient versus controls group. Also, high significant decreased (p=0.022) was found between mild preeclamptic group versus control pregnant group also, there was highly significant decreased (P=0.002) between severe preeclamptic group versus control pregnant (Table1-2). But no significant difference (P=0.062) between mild against sever group showed Table (1-2).

Table 2: The results of the serum placental protein 13 (pp13), urine placental protein 13 (pp13) for both control group and PE groups (mild and severe cases) at 3rd trimester.

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SE</th>
<th>P value</th>
<th>Control vs Mild</th>
<th>Control vs Sever</th>
<th>Mild vs Sever</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum pp13 (pg/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>67.06±3.296</td>
<td>0.006*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PET</td>
<td>54.29±3.129</td>
<td>0.036*</td>
<td></td>
<td>0.035*</td>
<td>0.900 NS</td>
</tr>
<tr>
<td>Mild</td>
<td>54.33±4.312</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sever</td>
<td>54.25±4.648</td>
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</tr>
</tbody>
</table>

Table 2: The results of the serum placental protein 13 (pp13), urine placental protein 13 (pp13) for both control group and PE groups (mild and severe cases) at 3rd trimester.

**Table 2**

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SE</th>
<th>P value</th>
<th>Control vs Mild</th>
<th>Control vs Sever</th>
<th>Mild vs Sever</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum pp13 (pg/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>67.06±3.296</td>
<td>0.006*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PET</td>
<td>54.29±3.129</td>
<td>0.036*</td>
<td></td>
<td>0.035*</td>
<td>0.900 NS</td>
</tr>
<tr>
<td>Mild</td>
<td>54.33±4.312</td>
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<td></td>
<td></td>
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<tr>
<td>Sever</td>
<td>54.25±4.648</td>
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</tr>
</tbody>
</table>

Table 3 showed comparison between placental protein 13 urinary and serum among preeclamptic and control women, the mean ±SE of serum levels preeclamptic and control were 54.29±3.129 pg/ml, 67.06±3.296 pg/ml respectively while the mean ±SE of urine levels for preeclamptic and control 38.39±2.717 pg /ml 51.84± 2.601 pg/ml when compared with serum pp13 between patients and control showed high significant (p=0.006). Also, patients compared urine pp13 was found with very high significant decreased (p=0.001)

Placental protein 13 urine and serum was measured in sera samples patients and healthy was presented in figure (3.2)
Table (3) Correlation of serum placental protein 13 pg/ml with all of the studied parameters in control pregnant group, mild and severe preeclamptic group.

<table>
<thead>
<tr>
<th></th>
<th>Serum pp13 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control pregnant</td>
</tr>
<tr>
<td>Age</td>
<td>-0.496**</td>
</tr>
<tr>
<td>Urine pp13 (pg/ml)</td>
<td>-0.008</td>
</tr>
<tr>
<td>pp13 ratio</td>
<td>0.694**</td>
</tr>
</tbody>
</table>

Regarding the correlation of the serum pp13 concentration with all of the studied parameters in control, mild, and severe PE groups; in severe PE, serum pp13 values showed very high positive significant correlation (P<0.001) with pp13 ratio=r=0.806). And positive correlation with urine pp13 (r= 0.019). On the other hand, in mild PE, positive correlation with age (r=0.096) In the control group, the highest level of significance (P<0.001) were observed in correlation of PP13 with age (r=- 496), and pp13 Ratio=r= 0.694). Also it showed significant negative correlation (P<0.01) with age, urine pp13.

Table (4) Test Result Variable

<table>
<thead>
<tr>
<th>Test Result Variable(s)</th>
<th>Area</th>
<th>cutoff</th>
<th>Sensitive</th>
<th>Specific</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum PP13 (pg/ml)</td>
<td>0.700</td>
<td>50,43</td>
<td>64%</td>
<td>82%</td>
<td>0.002*</td>
</tr>
<tr>
<td>Urine PP13 (pg/ml)</td>
<td>0.746</td>
<td>50,43</td>
<td>64%</td>
<td>82.5%</td>
<td>0.0001*</td>
</tr>
</tbody>
</table>

Discussion

Preeclampsia influences about 2 to 5 percent of women at pregnancy, causative to the fetal, neonatal and maternal mortality and morbidity, whilst the disorder noticeable at the 3rd trimester, the main placental function disturbances starts to a great extent in pregnancy earlier. Placental protein 13 (PP13) is a 32 kDa which is a dimmer protein that is present just in the placenta and is consider to be included in maternal artery remodeling and usual placentation.

Regarding ROC study between patients and control: Receiver operating characteristic curve (ROC) analyses of serum PP13 reveals the capacity of this biomarker to distinguish normal pregnancies from preeclamptic (Figure 3.5). The cut-off value = 50.43ng / ml of PP13 optimally identified patients with preeclampsia; at the sensitivity was 64% and specificity was 82.5 % and the area under curve (AUC) was 0.70 P = 0.002 .ROC study between patients and control : Receiver operating characteristic curve (ROC) analyses of serum PP13 reveal the ability of this marker to differentiate preeclamptic from normal pregnancies (Figure 3.5). The cut-off value = 50.43ng / ml of PP13 optimally identified patients with preeclampsia; at the sensitivity was 64% and specificity was 82.5 % and the area under curve (AUC) was 0.746 P = 0.002.
differences in the mean age of preeclampsia group and control normotensive.

In the current study, the maternal values of the placental protein 13 in serum were found highly significant in pre-eclamptic cases groups (mild and severe preeclampsia) as compared to controls group and showed decrease in the results of pp13 levels in preeclampsia disorder.

The result conducted by Fahmy there was low in pp13 levels in pre-eclampsia groups, this agreement with our study appeared there was decreases in pp13 levels in preeclampsia groups as compared with control pregnant group Perhaps, (Fahmy et. al. 2018).

And in agreement with Berthold Huppertz observation in patient with Preeclampsia the pp13 serum levels were increased compared with control pregnant individuals. (Berthold Huppertzetal, 2008)

And the study by Farina conclude that variations in PP13 in preeclampsia be able to be measured revealing a pathophysiological alter of PP13 in pregnancy very early. It stays to be observed if the decreased value of PP13 in pregnancy early is an essential reason or an outcome of the placentation disturbance (Farina et al., 2010).

Conflict of Interest – Nil
Source of Funding- Self
Ethical Clearance – Not required

References
Efficacy of Acetaminophen Plus Amitriptyline Compared to Acetaminophen to Reduce Pain Intensity in Nonspecific Chronic Lower Back Pain

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¹Resident in Training Department of Neurology, ²Department of Neurology, Faculty of Medicine, Universitas Airlangga Dr. Soetomo Teaching Hospital, Surabaya 60285, Indonesia

Abstract

Background: There was 90% of Lower back pain (LBP) are non-specific. Acetaminophen is the first analgesic in the treatment of LBP, but some cases of chronic LBP need additional antidepressant drugs. Amitriptyline is an antidepressant drug often used in cases of pain, but the evidence research in the case of non-specific chronic LBP is still a contradiction.

Method: The study was a Double-Blind Randomized Controlled Trial using consecutive sampling admissions. The subjects were divided into 2 groups; acetaminophen plus amitriptyline group and acetaminophen plus placebo group.

Results: There was no significant difference in pain intensity of acetaminophen plus amitriptyline and acetaminophen plus placebo groups either statistically or clinically (p = 0.498; OR = 0.667; CI 95% 0.20-2.16; ARR = -0.07 or -7%). The significant improvement of pain intensity in the treatment group Acetaminophen plus Amitriptyline was 24 (72.7%), while in the control group Acetaminophen plus Placebo was 24 (80.0%). In the treatment group was 9 (27.3%) who did not experience a significant improvement in pain intensity, and in the control group was 6 (20.0%).

Conclusion: There was no difference in efficacy between acetaminophen plus amitriptyline with acetaminophen plus placebo to reduce pain intensity in non-specific chronic LBP.

Keywords: Non-specific chronic LBP, Amitriptyline, Acetaminophen, VAS

Introduction

Lower back pain (LBP) is a health problem in adults that most often occurs in developing countries around the world. Approximately 90% of LBP patients are non-specific, and one-third of them have chronic symptoms about a year after an acute episode. According to the World health organization (WHO) International classification of functioning definition, disability, and health; nonspecific LBP is an unknown LBP that underlying pathology in the absence of tissue damage to corresponding symptoms of LBP. Acetaminophen is still the first-line analgesic recommended by almost all pain guidelines in the treatment of chronic LBP, but in some cases, additional treatment is required, such as antidepressants or anti-convulsions. Amitriptyline is one of the most widely used tricyclic antidepressant drugs as an adjuvant analgesic primarily for chronic neuropathic pain and fibromyalgia. Amitriptyline 25 to 125 milligrams each could reduce neuropathically and fibromyalgia pain Relative risk (RR) 2.3, (95% CI, 1.8-3.1) with number needed to treat 4.6 (3.6-6.6), however 64% of participants in the study experienced adverse events RR = 1.5, (95% CI 1.4-1.7) with needed to harm
4.1 (95% CI 3.2-5.7). Another result mentioned that in the case of temporomandibular joint pain, amitriptyline at a dose of 10-30 milligrams/day was effective in relieving pain with pain reduction occurring within 6 weeks.

In addition, there were other objectives such as: Assessing the intensity of pain in chronic non-specific LBP patients by receiving acetaminophen 3x500 milligrams daily; Assessing pain intensity in non-specific LBP patients chronically treated by acetaminophen 3x500 milligrams plus amitriptyline 10 milligrams daily; Comparing pain intensity changes in chronic non-specific acetaminophen LBP patients by receiving acetaminophen 3x500 milligrams plus amitriptyline 10 milligrams daily with non-specific chronic LBP patients that receiving acetaminophen 3x500 milligrams. Therefore, the study aimed to prove that acetaminophen 3x500 milligrams plus amitriptyline 10 milligrams each day was better in reducing the intensity of pain than the administration of acetaminophen 3x500 milligrams each day in non-specific chronic LBP.

Method

Double-blind randomized controlled trial was used in the study. The subjects were non-specific chronic Lower back pain (LBP) patients at the Outpatient Neurology Unit of Dr. Soetomo Teaching Hospital Surabaya that fulfilled the criteria of inclusion and exclusion. The inclusion criteria were; the samples have not received treatment yet, Not treatment anti-pain drugs within a week before joining the study, Age ranges of 20-49 years, Visual analogue scale (VAS) ≥4. And willing to participating in the research by sign informed consent. Whereas, the exclusion criteria were: Depression, such as the heart is relatively unknown and noticed by radiographers and cardiologists. Objective: To analyse the image quality of 4 chamber sections of Cardiac Magnetic Resonance Imaging with and without the use of shim volume on Steady State Free Precession (SSFP. Data on the intensity change of pain was obtained from the difference between before and after the treatment, then it will be analyzed using a Chi-square test (X2). The preliminary study of 30 people was obtained 80% of the subjects that had experienced a treatment success (VAS1-VAS2 ≥2 difference) on the 14th day of treatment, so the cut point taken of treatment was 13 days.

Results

Tabel 1: The Characteristics of Baseline Age Data in Treatment and Control Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment (Asetaminofen +Amitriptilin)</th>
<th>Control (Asetaminofen+Plasebo)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ages</td>
<td>Median - Minimal - Maximal</td>
<td>Median - Minimal - Maximal</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>42.00 - 20-49</td>
<td>37.50 - 20-49</td>
<td>0.524 (Mann-Whitney)</td>
</tr>
</tbody>
</table>
Table 2 The Characteristics of Research Subjects By Sex, Education Level, and Occupation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>Kontrol</th>
<th>Total</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>26 (55.3%)</td>
<td>21 (44.7%)</td>
<td>47 (100%)</td>
<td>0.424</td>
</tr>
<tr>
<td>Male</td>
<td>7 (43.8%)</td>
<td>9 (56.3%)</td>
<td>16 (100%)</td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Junior High School</td>
<td>7 (41.2%)</td>
<td>10 (58.8%)</td>
<td>17 (100%)</td>
<td>0.279</td>
</tr>
<tr>
<td>Senior High School</td>
<td>26 (56.5%)</td>
<td>20 (43.5%)</td>
<td>46 (100%)</td>
<td></td>
</tr>
<tr>
<td>Jobs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moving Around</td>
<td>19 (48.7%)</td>
<td>20 (51.3%)</td>
<td>39 (100%)</td>
<td>0.458</td>
</tr>
<tr>
<td>Stay Still</td>
<td>14 (58.3%)</td>
<td>10 (41.7%)</td>
<td>24 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 The Characteristics of Pain Intensity Visual analogue scale (VAS) of the Initial Research Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment (Asetaminofen+Amitriptilin)</th>
<th>Control (Asetaminofen+Plasebo)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (minimal-maximal)</td>
<td>Median (minimal-maximal)</td>
<td></td>
</tr>
<tr>
<td>Early VAS</td>
<td>5.00 (4.20-8.50)</td>
<td>5.20 (4.10-8.20)</td>
<td>Mann-Whitney 0.540</td>
</tr>
</tbody>
</table>

Table 4 The Changes in Pain Intensity in The Treatment and Control Group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment (Asetaminofen+Amitriptilin)</th>
<th>Control (Asetaminofen+Plasebo)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Standard Intersection</td>
<td>Mean Standard (IK95%) Intersection</td>
<td></td>
</tr>
<tr>
<td>Difference of</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAS1</td>
<td>3.50</td>
<td>2.09</td>
<td>0.28</td>
</tr>
<tr>
<td>VAS2</td>
<td></td>
<td>4.07</td>
<td>-1.16 0.47 (IK95%)</td>
</tr>
</tbody>
</table>
Basic Demographic and Clinical Data of Subject

The basic characteristics of subjects by age (Table 1) showed that the median age in the treatment group Acetaminophen plus Amitriptyline was 42.00 (20-49), while in the control group Acetaminophen plus Placebo was 37.50 (20-49). The homogeneity test of the age variables in the two groups (Table 1), showed no significant difference age between treatment and control group with Mann-Whitney p = 0.345.

Characteristics of basic data of research subjects based on sex, educational level and type of job are shown in Table 2. Overall, there were 47 females and 16 males. The treatment group was 26 (55.3%) of female subjects and 7 (43.8%) of male subjects.

Clinical data of study subjects before treatment shown in Table 3. In the treatment group, the median data of initial VAS was 5.00 (4.20-8.50), whereas in the median control group the initial VAS was 5.20 (4.10-8.20). The homogeneity test of early VAS variables in both groups had no significant difference in initial VAS variable between treatment and control group, with Mann-Whitney p = 0.540.

Effect of therapy on the improvement of pain intensity Changes in pain intensity were the differences between the initial Visual analogue scale (VAS), on day 14th (VAS1-VAS2 differences). The mean of pain intensity change in the treatment group was 3.51±2.01, and in the control group was 4.08±2.05 (Table 4).

The test of variable distribution of pain intensity change in both groups showed an abnormal distribution pattern, so the comparative test used to compare the variety of pain intensity change in both groups using an unpaired t-test. The unpaired t-test of VAS1-VAS2 differences between the two groups showed no statistically significant difference, with p = 0.28 (CI 95%
Result Data based on the success of treatment

A significant improvement in pain intensity was considered successful, for example, the initial VAS value difference and the VAS day value of 14 (VAS1-VAS2 difference) was ≥2. Overall, 48 (76.2%) of the subjects who succeeded in the treatment (decreased pain intensity ≥2) and 15 subjects (23.8%) were unsuccessful in the treatment. The significant improvement of pain intensity in the treatment group Acetaminophen plus Amitriptyline was 24 (72.7%), while in the control group Acetaminophen plus Placebo was 24 (80.0%). In the treatment group was 9 (27.3%) who did not experience a significant improvement in pain intensity, and in the control group was 6 (20.0%). The result of the statistical test comparing the treatment success between the treatment group and control group was not statistically significant, with p = 0.498; Odds Ratio = 0.667 (CI 95% 0.20-2.16).

Drug Side Effects in the treatment and control group

During the study, the side effects were found in both control and treatment groups, with results as shown in Table 6. The incidence of adverse drug effects in the study subjects of treatment group was 21 people (63.6%), whereas in the control group was 15 (50.0%).

Discussion

The study, there were no significant differences in both the outcome of decreased pain intensity and treatment success, between the treatment group and the control group. It was not following previous studies suggesting that amitriptyline was more effective in the second week of treatment in reducing pain intensity (t = 4.43, p <0.001) than acetaminophen (t = 3.30, p <0.01) LBP 45. Some of the reasons that might explain the difference between our study and previous research were the inclusion criteria were non-specific LBP cases that exclude depression, did not take into account psychological factors (anxiety and coping mechanisms), and use low doses of Amitriptyline.

Another Result included both non-specific LBP and neuropathic LBP in the inclusion criteria, whereas in the study subjects were only non-specific chronic LBP. They also take into account psychological factors such as mild depression, anxiety, and coping mechanisms.
treatment and the control groups, were no significant differences. The clinical trial, ARR = -0.07, indicated that statistically and clinically, treatment in the treatment group (Acetaminophen 3x500 mg + Amitriptyline 10 mg) was no improvement in reducing pain intensity than in the control group.

The incidence of adverse events in the treatment group and the control group was also differed. Administration of acetaminophen 3x500 mg plus amitriptyline 10 mg resulted in greater adverse events compared to the control group (Odds ratio: 1.750), but the difference between the two groups was not significant. This suggests that the addition of Amitriptyline at a dose of 10 mg of acetaminophen might have a greater side effect than single acetaminophen in a case of non-specific chronic LBP.

Another study supporting the results of our study was a meta-analysis of tricyclic antidepressant drug administration compared to placebo in the case of chronic non-specific LBP. In addition, a systematic review of the use of antidepressants in chronic LBP cases suggests that tricyclic antidepressants were superior only mild to moderate when compared with placebo on pain intensity output (SMD = 0.43 and SMD = 0.69 in 2 high-quality studies). The mean superior mild to moderate decrease in pain intensity was 0.5-2 points, whereas in our study considered to be significantly improved the pain intensity that was decreased ≥2.

The work of Amitriptyline as an analgesic to strengthen the pathway of descending inhibition by increasing the number of noradrenaline and serotonin neurotransmitters in the synaptic gaps at the spinal and supraspinal levels, especially, nor-adrenaline uptake. There were several possible reasons why the administration of acetaminophen 3x500 mg plus Amitriptyline 10 mg unproven to be better than the administration of acetaminophen 3x500 mg. The first possible mechanism for the onset of pain in non-specific chronic LBP was more due to the release of excessive inflammatory mediators, and not due to reduced levels of Noradrenaline and serotonin neurotransmitters at the supraspinal level, as have excluded depression in the study. Moreover, the possibility at a dose of 10 milligrams Amitriptyline has not reached its optimal dose as an analgesic.

**Conclusion**

There was no difference in efficacy of Acetaminophen 3x500 mg plus Amitriptyline 10 mg with Acetaminophen 3x500 mg in reducing pain intensity in non-specific chronic Low back pain (LBP).

**Conflict of Interest:** There is no conflict of interest.

**Source of Funding:** This study is self-funded.

**Ethical Clearance:** This study was approved by Ethical Commission of Health Research Faculty of Medicine University of Airlangga.

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Society Perception of Pasung Behavior in People with Mental Disorders

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Abstract

Introduction: Pasung intervention of people with mental disorder still happened in the society. “Pasung Free Program” which has already designed in Indonesia since 2014 did not effective to wipe out Pasung behavior. There are still many people who are abused by Pasung behavior in Kulon Progo, Java Island, Indonesia with the various perception of society. The aims of this study examined the public perception of Pasung behavior in people with mental disorders.

Method: This study used quantitative method by using symbolism interaction approach. There were 9 people who experienced pasung in their surroundings that became samples. Data was collected by using in-depth interviewing and socio-demographic questionnaire. Method that was used to analyze was analysis method such as 6 stages of Creswell analysis that was suitable with the used method.

Result: The result of this study could become a theme. There were 8 themes came from two different perceptions. Perception of society resulted two themes namely 1) opinion about pasung behavior and 2) conclusion about pasung. External perception resulted six themes namely 1) the reason why pasung is allowed, 2) the reason why pasung is not acceptable, 3) pemasungan idea, 4) pemasungan method, 5) the society’s hope about people with mental disorder, and 6) the obstacle of health service.

Conclusion: Society perception about mental disorder still not acceptable. Pasung program planning should be in line with free people with mental disorders program. The appearance of pasung behavior related to the increase of people with mental disorders.

Keywords : Pasung, Society perception, Mental Disorders,

Introduction

Savings are still often found in people with mental disorders in several countries with a various reasons that are brought up by families and communities around¹. Society misperceptions of people with mental disorders lead to negative stigma. Negative stigma arises due to the experience of patients who cause different treatment from the society ²,³. Discrimination due to stigma is one of the triggering factors for aggressive behavior of mental disorders’ patients, but the behavior is actually one of the reasons for retention that occurs in the society.

Keywords : Pasung, Society perception, Mental Disorders,

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The prevalence of mental health problems in Indonesia is 6.55% ⁴,⁵. People with severe mental disorders with age above 15 years in Indonesia reach 0.46% [4]. This means that there are more than 1 million people in Indonesia suffering from severe mental disorders. The number of people that is as many as 11.6% of Indonesia’s population experienced emotional mental disorders⁶. The prevalence of severe mental disorders based on the 2013 Basic Health Research (RiskeElementary Schoolas) which is 2.7 per mile is in Yogyakarta and Aceh.

Severe mental disorder is an individual who experiences orientation disorder, the reality of mood changes, personality, habits and habits of withdrawal. Disturbances in reality orientation trigger aggressive and dangerous behavior in the client such as harming oneself, others and damaging the environment. Stigma
on this problem has negative consequences not only for sufferers but also for family members and the surrounding community.

According to the latest data the prevalence of severe mental disorders in Indonesian population is 1.7 per mile. People with severe mental disorders are vulnerable to experience discrimination from the community, and not infrequently experience savings. Dismissal of people with mental disorders is an action taken by the society by means of being locked up, chained by their feet, put into wooden blocks so that their freedom is lost. The word pasung refers to physical restraint or confinement of perpetrators of crimes, people with mental disorders and who commit acts of violence that are considered dangerous.

Indonesia has launched the “Indonesia Pasung Free” program in 2014, however, the reality is still found pasung with various forms and reasons for mounting. The number of ODGJ experiencing savings throughout Indonesia reached more than 18 thousand people. Kulon Progo Regency with an area of 586.28 km2 and a population of 388,824 inhabitants is divided into 12 districts. Based on data from the Health Office of Kulon Progo Regency, there were 22 cases of mental disorders in Pasung in 2014, in 2015 it decreased to 8 because of outreach and pickup by related agencies. The latest data in 2016 found cases of re-embedding in people with mental disorders after getting treatment at the Kulon Progo Mental Hospital, Java Island as many as 2 people.

King Imogene’s theory of the concept model of human interaction, states human beings as a whole open system that consistently interacts with the environment with the aim of helping individuals and groups maintain their health. Human groups have an important role in the life process of society and the realization of mutual relations between individuals with one another. This study aims to determine people’s perceptions of pasung behavior in people with mental disorders. Public perception will give birth to different behaviors and stigmas for people with mental disorders that have proven to be still inherent in society today.

Method

2.1 Design of Study

Method that was used was qualitative method by investigating particular social problem. The approach of study used symbolic interaction base with qualitative approach method that was not only as a method to be a method of study, but also as a theoretical perspective conceptually. The place of study was conducted in Kulon Progo, Daerah Istimewa Yogyakarta.

2.2 Sample of Study

Sample was used constructive sample. Collecting sample data used purposive sampling technique. There is no limit to the number of samples selected to obtain data saturation, so that 9 participants are selected. These are selected according to inclusion criteria.

2.3 Instrument of Study

The instrument used in the collection process is to use in-depth interview guidelines (in-depth interview), with the method of face to face interview, field notes / field notes (recording data obtained during interviews): such as participant and other expressions and recorders or voice recorders in the form of voice recorders MP3. The validity of the recording device is carried out using a trial recording the researcher’s voice.

2.4 Procedure of Study

Data were analyzed using data using significant statement analysis, the formation of units of meaning and the development of the essence of description. This research uses Creswell’s analysis method with 6 steps of analysis.

Result

Participants in this study were 9 participants, consisting of 5 women and 4 men. The age of participants is in the range of 30 to 65 years. Characteristics of participants can be seen from the table below:
Table 1 characteristic of participants

<table>
<thead>
<tr>
<th>Participant Initial</th>
<th>Age</th>
<th>Sex</th>
<th>Last Education</th>
<th>Religion</th>
<th>Job</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>43 years old</td>
<td>Female</td>
<td>Diploma</td>
<td>Moslem</td>
<td>House Wife</td>
</tr>
<tr>
<td>P2</td>
<td>30 years old</td>
<td>Female</td>
<td>Bachelor</td>
<td>Moslem</td>
<td>Carrier</td>
</tr>
<tr>
<td>P3</td>
<td>43 years old</td>
<td>Female</td>
<td>Elementary School</td>
<td>Moslem</td>
<td>House Wife</td>
</tr>
<tr>
<td>P4</td>
<td>58 years old</td>
<td>Female</td>
<td>Elementary School</td>
<td>Moslem</td>
<td>Businessman</td>
</tr>
<tr>
<td>P5</td>
<td>62 years old</td>
<td>Male</td>
<td>Elementary School</td>
<td>Moslem</td>
<td>Businessman</td>
</tr>
<tr>
<td>P6</td>
<td>50 years old</td>
<td>Male</td>
<td>Junior High School</td>
<td>Moslem</td>
<td>Staff</td>
</tr>
<tr>
<td>P7</td>
<td>49 years old</td>
<td>Female</td>
<td>Senior High School</td>
<td>Moslem</td>
<td>Farmer</td>
</tr>
<tr>
<td>P8</td>
<td>54 years old</td>
<td>Male</td>
<td>Senior High School</td>
<td>Moslem</td>
<td>Carrier</td>
</tr>
<tr>
<td>P9</td>
<td>65 years old</td>
<td>Male</td>
<td>Elementary School</td>
<td>Moslem</td>
<td>Farmer</td>
</tr>
</tbody>
</table>

The majority of participants had low education, namely as many as 4 people, 1 person with junior high school education, 2 people with high school education, and the remaining 2 participants took education at the University. All participants also worked in the informal sector, namely as many as 2 farmers, 3 people working in the private sector, 2 people as housewives, and the remaining 1 person as village apparatus. In general, all participants are in middle to lower economic conditions.

**Thematic Analysis**

The result of study showed that 6 themes which came from 2 perceptions: internal perception and external perception.

<table>
<thead>
<tr>
<th>Perception Types</th>
<th>Theme</th>
<th>Subtheme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internal Perception</td>
<td>Opinion about pasung behavior</td>
<td>Disobey law</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Abuse</td>
</tr>
<tr>
<td></td>
<td>The agreement of pasung behavior</td>
<td>Agree</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Disagree</td>
</tr>
<tr>
<td>External Perception</td>
<td>The reason of pemasungan</td>
<td>Violence</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Give up</td>
</tr>
<tr>
<td></td>
<td>Pemasungan Idea</td>
<td>Not disturb</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Family</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Society</td>
</tr>
<tr>
<td></td>
<td>Pemasungan Method</td>
<td>Used wooden</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Used chain</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Build and isolated house</td>
</tr>
</tbody>
</table>
4.1 Internal Perception

Self-perception is a statement that is directly sourced from within the participant. All participants gave answers to their opinions about pasung to people with mental disorders.

Opinion of Pasung Behavior

The first theme is pasung behavior opinion. This theme consists of two sub-themes, namely breaking the law and inhumane. The following participant statements related to sub-themes violate the law:

“If from the government, saving is not allowed ...” (P6)

«Indeed, pasung is a violation ...» (P8)

The inhuman subtheme is described as follows:

“I don’t want, so sorry. Pasung is not a good choice. It is so terrible and pity....”(P5)

Agreement with Submission Actions

The second theme is agreement with actions to protect people with mental disorders. This theme consists of two sub-themes, namely agree and disagree. The following participant’s statement on the sub-theme agrees:

Society draws conclusions about saving people with mental disorders based on what is in their minds. 7 participants argued that saving can be done, 7 participants also said they did not agree if it was done mounting. This can be seen in the following participant’s statement:

That was before; if fortunately it really raged our show as a family cannot overcome ... (P1)

“If people with mental disorders are not locked up, so it supposed to detrimental a healthy family......”(P9)

The sub-theme does not agree with the statement as follows:

“Do not agree, that’s hmm, she/he would move a lot so if put in pairs, told to be released, fortunately that, screaming continued ...” (P4)

“Yes, it is depicted, yes, we ourselves are confined in the house even though the room is saturated, doesn’t have any understanding, it can’t get out, right? It’s getting more sick .....” (P6)

4.2 External Perception

This perception is given by participants due to stimulation from outside the individual self. All participants gave answers to their opinions about pasung to people with mental disorders.

The Reason of Pemasungan

The first theme is about the reasons for saving. This theme consists of the sub-themes of violent behavior, despair, not disturbing. The following is the participant’s statement on the subtheme of violent behavior:

“It was the one who threw the stone tossing the house, hah [while his hand was drawing] ……. (P1)

“I was cheated, there is no something wrong, I was hit, I was given ashes while cooking rice. [trying to imitate] ...” (P6)

Desperate sub-themes are described in the following statements:

“So I ask that the family there is already a big little giving up (more or less give up) so, I told the children too, how?? Wow, please repent, please, please (it’s already repent, whatever), we can called it is in the sense of sincere right...... (P8)

The unobtrusive sub-theme is described in the following statement:

“I agree that’s because, what is that ... so as not to disturb the neighbors, the community can be calm. If someone is thrown by a stone then the house is thrown, if there are those children being chased ...”(P4)

The Idea of Pemasungan

The second theme is the idea of retention. This sub-theme consists of two sub-themes, namely community and family. The following statements from the community on community themes:

Yes her husband, because it damages ... (P3)

“Her husband was beaten, beaten by her husband, kept looking for people to install beams ...” (P5)

Community sub-themes are presented in the following statements:

“Straight to me, I said if I was locked up. If it goes mad, if I go on a rampage I will be beaten (hit) like that...
Pemasangan Method

The third theme is about the mounting method. This theme consists of 3 sub-themes using wooden blocks, using chains and locked in a separate room. The following statement of participants through the subtheme using wooden blocks:

“Yes, we use wooden randhu then split it to pinch the legs [while the participants’ hands are modeling] ...” (P4)

The community sub-theme uses the chain described by the participants in the following statements:

“As far as I know, I was chained, fastened (roped) and then roped in a pillar (large pole) on a pole inside the house ...” (P6)

“If you want to release the angel, right, that’s why before it is chained before the room is made. And that keeps the fatigue or goes away ... “(P9)

Community sub-themes confined in the room are described by participants in the following statements:

“Continue to build a house, use a bamboo wall spindle [smiling] ...” (P5)

Discussion

The opinion of the participants found in this study was that the response was responded to as an inhumane act for them. In accordance with the concept of Imogene King’s theory that the individual was an open system which one of the elements consists of perception. Participants observed using the five senses, experience and conclude.

In accordance with human rights, everyone has the right to receive the same treatment without discrimination. The act of retrieval will cause a person to lose his rights in various ways. Perspective on humanity perspective also causes an increase in stressors because of being locked up for a long time. The majority of acts of violence experienced by a person will be represented in the form of negative emotional experiences, so it was not uncommon for people with psychiatric disorders to be incarcerated more easily.

The social system defined the authority or authority as well as active authority in the social system the government has the authority to set policies in accordance with the applicable laws and regulations. Prohibition of people with psychiatric problems and people with mental disorders was contrary to the 1945 Constitution of the Republic of Indonesia: Article 28G paragraph (2) “Everyone has the right to be free from torture or treatment that degrading human dignity and is entitled to political asylum from other countries. From this situation caused families and communities to feel that health services do not provide a way out for mental health problems experienced.

Communication caused interpersonal systems that are formed by interactions between humans. In the process the community and family interact with each other so that a decision was made for mounting. This results in negative impacts especially for the mental as well as physical, psychological and also social patients. The physical impact caused by disruption of movement was the condition of the legs and hands will shrink, the muscles from the hips to the legs shrink because of the long time not used which will also affect the sympathetic nervous system. In addition, physical injuries also experience psychological and social impacts in the form of discrimination committed by the community.

Statements expressed by participants indicate that the health services obtained were less effective. Mental health services were still not a top priority although mental health problems continue to increase. Health workers, especially nurses as the main source of direct relations have a role in working with health institutions, consultants, resource providers, care providers, case managers, and helping with community education. The main focus in Community Mental Health Nursing (CHMN) is the importance of collaborating with families and communities.

It was hoped that there will be no more obstacles for the community and families to bring people with mental disorders to get appropriate treatment and care. For residents who do not get guarantees, the Regent Decree No.373 of 2016 concerning the participation of the poor beneficiaries of APBD contribution to the National Health Insurance Program in Kulonprogo Regency in 2017 can get free facilities (Regent’s Decree No. 373 in 2016).

Conclusion

Society perception towards mental disorders is not completely acceptable. Stigma and discrimination
towards people with mental disorders is more and more placed them as unable and isolated people. There is a big thing to be fixed by the Government than the pasung release. Pasung release is not an easy thing but prevent ODGJ could be pasung again post did a treatment is difficult. Thus, this should need another alternative as the effort to press the number of people with mental disorders.

**Ethical Clearance:** This research has passed the ethics test with number 328-KEPK by the health research ethics commission of the Faculty of Nursing, Universitas Airlangga, Surabaya, Indonesia.

**Conflict of Interest:** The author reports no conflict of interest of this work.

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**References**


Effects of the Use Knee-Ankle-Foot Orthosis on Proprioception Function Change in Healthy Adult Male

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Abstract

Background: Proprioception includes joint position and motion. Sensory information, central processes, and neuromuscular control are important in improving joint stability. The effective use of knee joint orthosis on knee joint performance, balance and coordination is still highly controversial and has slight information on the effect of the orthotic use on proprioceptive function.

Objective: To analyze the effect of knee orthosis on proprioception function in adult male health’s subjects.

Method: This study used an experimental research with pre-post study design. The sample of this study was the male patient of Physical and Rehabilitation treatment that met inclusion criteria. The data was taken by consecutive sampling in February 2017. The subjects were 11 healthy male adults who aged of 21-40 years old that meeting the inclusion criteria. All the subjects were measured proprioception function before and at 30 minutes using knee orthosis. Joint position sense (JPS) and time to detect passive movement (TTDPM), were measured by the cybex isokinetic dynamometer tool to measure the function of proprioception.

Result: All the mean values of JPS before and at 30 minutes by using orthosis on both sides did not statistically show a significant difference (p > 0.05), except at the corner of the JPS 60° on the right-sided knee (p < 0.05). It meant TTDPM before and during 30 minutes using orthosis on both sides of the knee also did not show a significant difference (p > 0.05).

Conclusion: The use of ambulatory knee-ankle-foot orthosis caused the change in proprioception (JPS) at the right side 60° knee angle on the subject before and at 30 minutes using knee orthosis.

Keywords: Healthy Adult Male, Knee Orthosis, Proprioception.

Introduction

Knee joint is the largest joint in the human body and ginglymus-type joint (hinge joint modifications) ¹. Knee motion and stabilization is performed by the origo muscles above the hip joint, the entire shaft of the femur is also above the knee joint of the lower limb muscles. Proprioceptive comes from the position of the body, refers to the sense of knowing the position of a person’s body that is classically composed of static and dynamic components ².³. The design of orthosis is used based on the desired function. Knee orthosis is either a sleeve or has a rigid bars design on one side or both sides, nonelastic hinges and straps ⁴. Knee orthosis types which are often used including prophylactics, rehabilitative, functional, unloader/Offloader ⁵.

The integrated set of various mechanoreceptor, nociceptors and afferent muscles is known as proprioception. This information allows for feedback for motor movement control through position sensation and motion sensation (kinesthesia) and provides dynamic stability of joint. The motor movement control achieved through the feedback mechanism is realized during movement, well monitored, as well as to the appropriate response to the feedback given. The wide variability of joint position sensation has been observed in some subjects. The presence of deficits in proprioception

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is associated with the increased frequency of injuries and recurrent injuries to the knee joint. Proprioception includes joint position and joint motion. Sensory information, central process, and neuromuscular control are important in improving joint stability.

The successful use of knee orthosis has been discussed over the years. Although there is evidence to suggest that orthosis can decrease the frequency of knee injury, some researchers have reported that in the use of knee orthosis there is no difference in the injury pattern. This problem is related to multifactorial epidemiological studies that are considered to be the cause of conflicting findings. These factors include the material components of the orthosis used, the different surface contact areas of each individual against the orthoses used. Researchers who tested kinematic joint and muscle activity have compared the condition without orthosis by using orthosis and observed differences in electromyographic and kinematic joint activity while performing the functional activity. Based on these findings, proprioception may be affected by the use of orthosis. Although there is still minimal information about proprioceptive testing and athletic orthoses, as well as groups of subjects with knee joint arthritis, there is an increase in proprioception function in the use of orthosis drytex economy hinged knee brace and hinge buttress orthosis. Similarly, in studies conducted, it was found that the use of orthosis can improve the function of healthy individual proprioception.

The effectiveness of the use of knee joint orthosis on knee joint performance, balance and coordination is still highly controversial and has the slight information on the effect of the orthotic use on proprioception function. At present, the proprioceptive function gets a great attention on the literature of sports medicine. In the study, the use of orthosis can improve the proprioception function, thereby increasing the mechanical stability of knee joint and the use of knee orthosis in athletes based on the factors which have implication for the proprioception function. Until now, there has been several researches on the effects of knee joint orthosis on proprioception function. This study was conducted to determine the effect of knee joint orthosis on proprioception function in healthy adult male.

**Method**

The sample of this study was a male patient of Physical and Rehabilitation treatment that meets inclusion criteria. The data were taken by consecutive sampling in February 2017. The inclusion criteria were healthy men, aged 21-40 years, the range of knee joint movement 0-135° in extension-flexion, no deformity and willing to sign informed consent. The subjects exclusion criteria were with lower extremities, pelvic and spinal injuries, underwent spinal, pelvic, knee and ankle joint surgery, with neurologic and systemic disease affecting the lower extremities, hyperlaxity, and unstable joints. The subject had informed consent and underwent a proprioception with Cybex Isokinetic Dynamometer, before and after 30 minutes using knee orthosis on both knees. This research was a pre-experimental research and posttest study design.

Joint position sense (JPS) and time to detect passive movement (TTDPM) were measured for proprioception using Cybex Isokinetic Dynamometer. The behavior examination in a sitting position with eyes closed and ears, flex knee at 90 degrees. JPS was measured at 30°, 45°, and 60°. JPS and TTDPM for both knees were measured before and after 30 min using knee orthosis. Then, an examination of JPS and TTDPM on both knees without and when using knee orthosis was collected and analyzed data by using t-pair test (Paired t-test). In this study used statistical tests SPSS V.20.0 (SPSS, Inc., Chicago, IL.) with a significance level of p <0.05.

**Results**

The total sample of characteristics subjects was 11 male. The mean age of the study subjects was 32.82 ± 2.99 years, with the lowest age of 28 years and the highest 36 years. The average height of subjects was 165.81 ± 6.03 cm and average weight were 66.18 ± 7.80 kg. The mean body mass index (BMI) was 24.25±3.11 kg/cm² (Table 1). In table 2 the JPS was explained at an angle of 30°, 45o, 60°

The mean difference of right-sided knee TTDPM without orthosis and at 30 min using orthosis was not statistically significant, as did the mean difference of TTDPM on the left side of the knee. The mean TTDPM without the use of orthosis on the right side knee and on the left side of the knee and also at 30 min using orthosis on the left side knee and right-sided knee also did not show statistically significant differences as seen in table3.
### Table 1. Characteristics of the Subjects

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y/o)</td>
<td>11</td>
<td>28.00</td>
<td>36.00</td>
<td>32.8182</td>
<td>2.99393</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>11</td>
<td>58.00</td>
<td>84.00</td>
<td>66.1818</td>
<td>7.80792</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>11</td>
<td>156.00</td>
<td>177.00</td>
<td>165.8182</td>
<td>6.03023</td>
</tr>
<tr>
<td>BMI (kg/cm²)</td>
<td>11</td>
<td>21.81</td>
<td>32.30</td>
<td>24.2500</td>
<td>3.11068</td>
</tr>
</tbody>
</table>

### Table 2. JPS on the corner $30^\circ$, $45^\circ$, $60^\circ$

<table>
<thead>
<tr>
<th></th>
<th>Right</th>
<th>p-price*</th>
<th>Left</th>
<th>p-price*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 JPS at an angle $30^\circ$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ without orthosis (°)</td>
<td>2.33±1.47</td>
<td>0.978</td>
<td>2.42±1.32</td>
<td>0.909</td>
</tr>
<tr>
<td>Δ 30 minutes with orthosis (°)</td>
<td>1.39±1.26</td>
<td>0.434</td>
<td>1.21±0.85</td>
<td>0.736</td>
</tr>
<tr>
<td>p-price * without orthosis and at 30 minutes using orthosis</td>
<td>0.163</td>
<td></td>
<td>0.078</td>
<td></td>
</tr>
<tr>
<td>JPS at an angle $45^\circ$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ without orthosis (°)</td>
<td>2.18±2.46</td>
<td>0.242</td>
<td>2.42±2.86</td>
<td>0.088</td>
</tr>
<tr>
<td>Δ 30 minutes with orthosis (°)</td>
<td>1.81±2.03</td>
<td>0.141</td>
<td>1.45±1.57</td>
<td>0.156</td>
</tr>
<tr>
<td>p-price * without orthosis and at 30 minutes using orthosis</td>
<td>0.215</td>
<td></td>
<td>0.250</td>
<td></td>
</tr>
<tr>
<td>JPS at an angle $60^\circ$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ without orthosis (°)</td>
<td>2.87±2.91</td>
<td>0.712</td>
<td>2.02±2.27</td>
<td>0.625</td>
</tr>
<tr>
<td>Δ 30 minutes with orthosis (°)</td>
<td>1.54±1.93</td>
<td>0.221</td>
<td>1.84±1.73</td>
<td>0.622</td>
</tr>
<tr>
<td>p-price * without orthosis and at 30 minutes using orthosis</td>
<td>0.018</td>
<td></td>
<td>0.631</td>
<td></td>
</tr>
</tbody>
</table>

Note: Δ is the difference between the reproduced angle of the subject with the angle of $30^\circ$, $45^\circ$, $60^\circ$ knee flexion. * p significance level tested by paired t-test (p <0.05).
Table 3. The average value of TTDPM

<table>
<thead>
<tr>
<th></th>
<th>Right</th>
<th>p-price*</th>
<th>Left</th>
<th>p-price*</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTDPM without orthosis (dt)</td>
<td>2.59±1.14</td>
<td>0.971</td>
<td>2.61±1.03</td>
<td>0.941</td>
</tr>
<tr>
<td>TTDPM 30 minutes with orthosis (dt)</td>
<td>2.22±0.93</td>
<td>0.415</td>
<td>2.33±1.26</td>
<td>0.364</td>
</tr>
<tr>
<td>p-price * without orthosis and at 30 minutes using orthosis</td>
<td>0.092</td>
<td></td>
<td>0.284</td>
<td></td>
</tr>
</tbody>
</table>

Note: the average value is the average time when the subject feels the knee passive movement. Significance level tested by paired t test (p <0.05).

Discussion

The assessed parameters were the JPS for a sense of joint position and TTDPM to assess the ability to detect passive movement of the joints. This examination was performed in one visit. Prior to the examination of JPS and TTDPM, firstly evaluated vital signs (blood pressure, pulse, and breathing) and measuring height, the weight of clinical examination of knee joint stability and knee extensor muscle strength with manual muscle test. Measurements of JPS and TTDPM were performed before the use of knee orthosis and at 30 minutes using knee orthosis. During the wait, research subjects can perform activities in the polyclinic. JPS measurements were performed at angles 30°, 45°, and 60°. TTDPM checks were done at 10/sec. The average measurement of each subject was taken from 3 times the measurement. The average JPS was calculated from the average value of the difference between the reproduction angle at a predetermined angle. The examination was performed on both knees on both the left and right sides.

Based from the results of this study, there were no significant statistically on proprioceptive differences in JPS and TTDPM in the right and left sides of the knee before using knee orthosis and at 30 minutes using knee orthosis except for right-sided knee at treatment angle 60°. On the right side of the knee at an angle of 60°, there was a statistically significant JPS. The study in this study is similar to the study of prophylactic knee orthosis conducted in the study used the prosthetic-type prosthesis knee of Mc David Knee Guard. The study was conducted on 36 healthy male subjects who performed the measurement of proprioception function before and when using knee orthosis, the result did not get a significant difference to the JPS. In the study, it was concluded that prophylactic knee orthosis had minimal influence on the proprioceptive feedback mechanism.

In the study was obtained on 24 healthy subjects (14 male and 10 female) to determine the effects of prophylactic knee orthosis on balance, proprioceptive, coordination and the strength of muscle. They use 5 different types of knee orthosis that will be used entirely by the subject. From this research, it was obtained that Drytex economy hinged knee brace has the best result for improvement of proprioceptive function compared to the other four orthosis. This type of orthosis was what we use in this study. In the study also reported that proprioceptive knee joints increased when using elastic neoprene in healthy subjects. It was believed that the use of elastic neoprene as found in prophylactic knee orthosis may stimulate the skin during joint motion and put pressure on joint muscles and capsules. Afferent feeds from a number of receptors present on the skin, muscles, ligaments and joint capsules contribute to the proprioceptive mechanism in the knee joint overall. Most cutaneous receptors will respond to changes in movement and quickly adapt. The difference in the results of this study with the findings in this study may be due to neoprene wrapping in knee orthosis is not completely appropriate around the knee joints thus reducing the increase in skin stimulation. In addition, in this study, we used healthy subjects without a history of problems in the knee joints to allow for statistically significant results.

On JPS examination of the knee joint, the target magnitude of the reliable flexion angle for the increase of the JPS in the vertical position was between the 60°-90° flexion angle. Target this knee joint position to get maximum JPS knee joint. It was also obtained in our study that between JPS angle 60° on right leg side knee without using orthosis and with 30 minutes using orthosis there was a statistically significant difference. It was said that in this position it was possible to obtain a balance of both agonist and antagonist muscles and
the required minimal complex neurological processes thereby that will causing a minimal error rate.

In this study we found a statistically significant JPS difference in the right-hand side 60° knee angle, this may be because the right knee was the dominant knee of the study subjects, but we can’t confirm it. So far we have not obtained data on the difference in the function of proprioception on the dominant and non-dominant side in healthy subjects and also still lack research on the use of knee orthosis which assessed the proprioceptive function of the knee joint.

For athletes and non-athletes who have previously suffered an ACL injury will show a worse knee joint JPS than a healthy subject. Joint Position Sense (JPS) and TTDPM without using orthoses compared to the time 30 minutes using orthosis in our study did not show the statistically significant difference, except at the right side 60° angle of the knee. The 30 minute time may be less to get a meaningful change and this still needs further research.

The TTDPM examination in this study uses Cybex isоquant dynamometer which in its use must be moved first in the direction of flexion before the machine moves toward the extension so that the subject was able to recognize when the machine starts to move and it can also be biased in the examination.

Study Limitation

In this research, we were using healthy adult male subject without any problem at the knee joint so the result was not significant statistically and the study effect when subject underwent 3 times measurement before using orthosis so the subject have experience.

Conclusion

The use of knee orthosis for 30 minutes may improve the healthy knee joint proprioception function at the right side corner of the JPS 60°. The use of knee joint orthosis cannot improve the knee proprioception function in TTDPM at 30 minutes of application.

Conflict of Interest: There is no conflict of interest.

Source of Funding: This study is self-funded.

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References


The effectiveness of Aqueous Extract of Grape Seeds *Vitis vinifera* as an antibiotic for some microorganisms and its Protective Role Histology for Liver, Kidney in Mice

Osama Nadhom Nijris¹, Zinah Ibrahim Khaleel¹, Swiss Yonis Hamady¹, Mohammed Ahmed Mustafa¹

¹Department of Pathological Analysis, College of Applied Sciences, University of Samarra, Iraq

Abstract

1- Among the common disease treatments and long-term use of plant extract in the treatment of bacterial infections, This research used black grape seed extract to observe its effect on some Gram negative and positive bacteria like *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Proteus mirabilis* obtained from patients with urinary tract infection Using four specific extract concentrations (100 mm / L, 75 mm / L, 50 mm / L, 25 mm / L) to test the effect of black grape plant extract. The results showed that *E. coli* was the most resistant bacterial species to plant extract and so on with other gram negative bacterial species. In the minimum inhibition concentration test for the plant extract, showed that the highest concentration 100 mm / L had the good inhibitory activity for *Staph. aureus* and less than other strains specially for *Kleb. pneumoniae* and *Pro. mirabilis* while the 25mm / L concentration had the lowest activity against all.

2- This study used 10-day oral methionine and 30-day grape extract treatment for 16 white mice to study the histopathological effects in the liver, kidney and heart tissues, And the results showed a very good organ tissue improvement for all treated groups compared to the control group.

Keywords: bacteria, extract grape seeds aqueous, histopathological effects.

Introduction

Researchers have turned to plant extracts as natural products to tract bacterial infection and tissues damage because plants are rich with vital compounds such as volatiles oils, Tannins, Alkaloids, Phenols, Glycosides and Saponins (1).

Urinary tract infection is known as the presence of pathogenic microorganisms within the vessels of the urinary system so that they appear in the urine, *Escherichia coli* is responsible for causing about 80-90% of infections, and so on for all types of Enterobacteriaceae family, especially urethritis and bladder in Women and children, as for the positive group of gram stain, *Staphylococcus aureus* is the most common type of this type of infection (2),(3),(4).

Grape Seed Extract is one of the richest sources of powerful and beneficial component called flavonoids, the most important of which is Proanthocyanidin, which is 50 times stronger than the effect of vitamin such as C and E participate in protecting the body from the effect of oxidization by free radicals (5), and these Antimicrobial phytochemicals such as flavonoids have another functions such as Anti-inflammatory, anti-allergenic, anti-viral and anti-cancer action (6),(7). Also the Phenol which found in bear berry with an antiseptic urinary effect (8), Saponins also have anti-inflammatory, anti-microbial, anti-protozoan and immunostimulative properties (9).

As well as its effectiveness against bacteria, viruses and inflammatory diseases through the inhibition of peroxide formed by phagocytic cells (10).

For the above reasons, this research has came to study the effect of aqueous extract of grape seed on some bacterial species isolated from urinary tract infections and to study its effect of treatment for methionine treated tissues.

Materials and methods

**Bacteria diagnosis:** Samples were obtained from Samarra General Hospital’s urinary tract infections,
We have been identified through the routine procedures listed in Wise, A. (2017)\(^{11}\).

Four bacterial species were obtained: *Staph. aureus*, *E. coli*, *Kleb. pneumoniae* and *Pro. mirabillis*, which was predominant on most specimens of urinary tract infection and was subsequently taken as a test bacterial species for subsequent testing. And all its characteristics that were diagnosed as a result were identical to those mentioned in above reference\(^{11}\).

**Sterilization of the extract and preparation of fears:**

Prepare a storage solution by taking 1gm of dry plant extract powder and dissolved in 10 ml of sterile distilled water. The concentration of the storage solution is 100 mg / ml. 0.11mm filter papers were used to separate the large pieces of the extracted particles and the extract was sterilized by Millipore 0.22 mm which prevent the passage of contaminant bacteria through it to obtain a sterile storage solution of 100 mg / ml and use it as a source for preparation of fears\(^{12}\).

**Preparation of concentrations of grape seed extract:**

The four concentrations (25,50,75,100 mm/L) have been prepared through addition amount of storage solution to equal volume of distilled water as following equation:

\[
\text{Volume of storage solution/volume of distilled water.}
\]

**Assessment of antimicrobial potential:**

Neomycin (10 Mg) has been used in all media plates as a normal control for plant extracts. according to Bridson (2006)\(^{13}\), standard antibiotics and plant extracts were used in the “cylinder-plate” process to carry out the antibiotic sensitivity test. The test was performed by individually injecting extract solutions or standard antimicrobials into the media cylinders and measuring the inhibition zones by the zone reader system after incubation.

**Test for histopathological effects**

Methionine have a molecular weight of 472.09 and was dissolved in the solution to prepare a natural solution and was orally administered for a period of seven days at a concentration of 0.5 mg / kg twice a day\(^{14}\).

**Preparation of the water extract of grape seeds**

The water extract of grape seeds was prepared by method mentioned by Bayadar. N.G.\textit{et al.},\(^{15}\) by crushing a quantity of clean seeds using the Ultra-Tubax blender (Germany), Then, 50 g of dry powder was weighed and placed in a 1000 ml glass container Add the distilled water and complete the volume to 1 liter then add 3 ml of absolute ethyl alcohol to prevent fungal growth.

The samples were left for half an hour in the horizontal vibrator and at medium speed. The samples were stabilized for an hour, then filtered by three layers of gauze to separate the large plankton and then filtered to 3,000 centrifuges / min for 15 minutes to separate the small plankton. Of the extract at 40 °C using rotary evaporator and put in incubator at 37° C and then save the extract in a sealed bottle at a temperature of 8° C in wet conditions for use in the study.

![Figure 1 Black grape seeds](image)

**Laboratory Animals group:** In this study, 16 mice from Swiss white mice were used, ranging from 25 to 28 g. Obtained from Pharmacology Department / General Pharmaceutical Company for General Industries. The animals were in good health and were placed in laboratory plastic cages dedicated to the breeding of mice, and sprinkled with sawdust, with cages cleaned and sterilized twice a week. Group design: The animals were randomly divided into four groups in plastic cages. Each cage contained four mice, and treated as follows:

1- **Control group:** consists of 4 mice treated with normal saline solution, 0.1 mg/ kg

2- **Group I:** The First Group , Was composed of four mice treated orally with methionine 0.5 mg / kg for 10 days.

3- **Group II:** The Second Group , Was composed of four mice treated orally with aqueous extract of grape
4- Group III: The Third Group, Was composed of four mice treated orally with aqueous extract of grape seeds 30 % mg / kg for 30 days, as mentioned by AL.Jeboory et al., (16).

Preparation of tissue sections After dissecting the animals and placing organs in the Formalin 10% solution, the samples to be studied were converted to ethyl alcohol at 70% concentration. The following steps were taken as mentioned by Khaleel .Zinah I. et al., (17).

Result and Discussion

Assessment of antimicrobial potential:

Grape seed extract showed highest inhibitory activity on Staph. aureus, But to a lesser extent Kleb. pneumoniae and Pro. mirabillis bacteria which were 6mm at 100mm / L, 5mm at 75mm / L and 4mm at 50mm / L, But in general, the four concentrations of the extract had significant effect compared to the antibiotic Neomycin with gram positive group, whose average inhibition diameter for the four types of bacteria ranged between 10-20mm. These results were in agreement with what was stated by Jayaprakasha and. Sakariah (18), They indicated that grape seeds water extract have a greater effect on gram positive bacteria than negative, whereas 25mm / L concentration had the lowest inhibitory activity, which was 3mm for Staph. aureus, Pro. mirabillis, while it showed no effect on Kleb. pneumoniae and E.coli,

Figure 2: the effect of extraction on Pro. mirabillis

Figure 3: the effect of extraction on Kleb. pneumoniae

As for E. coli bacteria were resistant to all extract concentration, only the concentration of 100 mm / L caused inhibition of a diameter of 1 mm as shown in table 1.

Table 1 the inhibitory activity of four concentrations of black grape seed extract:

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Plant extract concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100mm/L</td>
</tr>
<tr>
<td>E. coli</td>
<td>1 mm</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>11 mm</td>
</tr>
<tr>
<td>Kleb. pneumoniae</td>
<td>6 mm</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>6 mm</td>
</tr>
</tbody>
</table>
The result that showed with table 1 referred that *E. coli* was the most resistant bacterium used in the study. This shows that it has developed its ability to resist antibiotics in contrast to the other species belong to both gram positive and negative, these results agree with Zdenka Cvetni and Sanda Vladimir (19), Whom made it clear the grape seed extract less affect on Enterobacteriaceae family like Salmonella enteritidis, although Gram positive bacteria, the extract showed the efficacy of its orientation not similar to that of the Gram negative bacteria and this result is not necessarily consistent with the result of the study done by Fleming (20), In his study, *Staph. aureus* showed that the bacteria were more strongly affected than the negative bacteria of Gram.

**Liver**

Histological sections of the first group of mice treated with methionine for 10 days showed tissue changes such as necrosis, degeneration, congesting, hemolysis, sinusoidal enlargement, and hepatocyte swelling. With Infiltration Lymphocyte lymphocytes and hemorrhagic hemorrhage, as well as pyknic nuclei.

The histological examination of the liver section in the animals treated with the grape extract for the second group for 30 days showed hemorrhage and lymphocytic infiltration with intracellular necrosis of the central vein (desquamation). A few cases of Karyolitic were also found with some nuclei that suffered small size and Pyknic Nuclei intensified and also found improvements in liver cells in terms of return to normal form compared to the group of methionine. While the results of the second and third group of the same period showed a good improvement of the hepatic cells and regularity of the natural form as it was approached the normal form, although there are some cases of hemorrhage bleeding and lymphocyte Infiltration lymphocyte. and the third group was better.
The liver tissue showed a significant effect of methionine at the concentration level, with bleeding, degeneration, lymphocytic infiltration, and endogenous degeneration and decomposition. These results were consistent with the findings of Al-Saidya, Al-Shammry, and Al-Okaily. Hepatotoxic substances are hydrogen peroxide and methionine. The toxicity of substances lies in their ability to form free radicals such as O-2 and OH. These roots are characterized by their ability to interact with lipid and proteins involved in the synthesis of membranes Kink and inside cellular and Intracellular membrane and thus discourage many vital special events such as the transmission of membrane necessary for the sustainability of cell materials also interfere with the action of enzymes and thus inhibit cellular respiration which leads to a reduction of cell events.

The interaction of free radicals with lipids results in lipid peroxides, which stimulate a series of membrane interactions with a decrease in mitochondrial membrane viability and destruction of the particle membranes of the Lysosomes and thus the cell’s arrival in the necrosis.

This study also showed the reform in the liver tissue of the methionine-affected cells and the return of these changes to the natural state using the water extract of the grape plant was due to the action of the extracts in inhibiting the action of methionine by possessing many activities, especially anti-oxidation and then speeding up the repair process And stimulate the tissue cells to secrete chemical attractants to attract inflammatory cells, which was clearly visible to the region for the purpose of feeding the damaged tissue, as well as stimulate the fabric to divide to compensate the affected by methionine.

**kidneys**

The examination of tissue microscopically showed that a section of the cells lining the urinary bulb is similar to normal, but in some places there is swelling in the proximal epithelial cells of proximal and distal proxies, as well as swelling of the nuclei There is general necrosis and vacuolated necrosis with Degeneration within a section of the glomeruli within its constituent cells with dense and small nuclei observed on their way to death.

Some cases of hemorrhage, lymphocytes and lymphocytes were observed, as well as minor bleeding within the glomerulus, lymphocytic infiltration into the glomerulus, and cirrhosis in the proximal and proximal tubules.

Methionine causes a significant increase in serum creatinine, urea nitrogen in the blood and uric acid concentration in the blood, and high blood urea nitrogen is a marker of kidney disorders.
Figure 8: section of the tissue of the kidney with a dose of methionine, showing (1) congestion (2) Vacuolated, 400X H&E.

Figure 9: kidney tissue, grape extract, group I, showing (1) glomerulitis (2) lymphocytic infiltration, H&E, 400X.

Figure 10: kidney tissue, grape extract, group III, showing (1) glomerular (2) lymphocytic infiltration, H&E, 400X.
The effect of methionine on kidneys is consistent with Dever and Elfarra (26), they pointed out that high levels of uric acid have been associated with gout, high blood pressure, kidney damage, hyper homocysteine in the blood, Blood to overproduction and release of oxygen species (ROS) from the glomerulus, and then kidney damage, double glomerular filtration rate,

The excess adenosine will interact with methionine which forms S-adenosel methionine and then degrade to form uric acid as a final product leading to hyperuricemia (27).

The results of this study revealed that excess methionine caused renal injury manifested in a significant deterioration in epithelial lining cells of renal tubules. These results are consistent with Rowan (28). Where glomerular lesion was observed and the progression of glomerular sclerosis was increased after methionine dosage.

As shown in this study, a severe renal dysfunction and this renal impairment may be due to homocysteine associated with increased adenosine level associated with increased level of sclerotic changes in glomeruli (28)

Ethical Clearance: It was obtained from the Scientific Research Committee at College of Dentistry/University of Babylon, Iraq.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

References


Comparison of Stability And Sternum Healing Rate in Clinical and Ultrasonography (USG) between Stainless Steel Wire and Polydioxanone Yarn in Children Heart Surgery Operation

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¹Thoracic Surgery, Cardiovascular and Vascular, ²Department of Radiology, Faculty of Medicine - Dr.Soetomo General Hospital, Universitas Airlangga, Surabaya 60285, Indonesia

Abstract

Background: The closing of the sternum bone is often used today by using stainless steel wire material and Polydioxanone yarn continuous suture. To objectives to be achieved for sternal healing after heart surgery without complications resulting from the failure of sternal healing. The bone healing process itself is influenced by mechanical stress and movement.

Objectives: To compare the effects of sternum closure techniques on surgical patients The heart of the child uses Polydioxanone and Stainless Steel Wire threads against clinical stability and rate of healing which are evaluated clinically and Ultrasonography.

Methods: Performed sternal closure of pediatric patients after cardiac surgery with sternal wire (n = 8) and PDS (n = 8). Performed sternal pain and stability evaluation with the physical examination. Further sternum ultrasonographyund was performed to assess displacement, gap and callus picture. Evaluations were performed at weeks 6, 9 and 12 postoperatively.

Results: Week 6 and 9 degrees of pain were higher in sternal wire compared with polydioxanone (p = 0.03 and p = 0.01). The 12th week of sternal wire and polydioxanone did not find any difference in pain (p = 1,000). Week 6, 9 and 12 there was no clinical stability difference between wire and PDS (p = 0.143, p = 0.264, p = 0.063). 9th, 9th and 12th Sternum ultrasonography of examination appears to be displacement in polydioxanone (p = 0.025, p = 0.009, p = 0.009). The gap increased significantly from 6th to 9th weeks in the polydioxanone group, while the addition at week 9 to 12 was statistically insignificant but it appears that polydioxanone had a wider gap addition range than the sternal wire. Week 9 and 12 callus were seen more often in sternal wire patients but not significant (P = 0.602, p = 0.333)

Conclusion: Clinically, sternal steal wire stability is proportional to polydioxanone. Radiologically, the stability of sternal wire is better than polydioxanone. The rate of sternal cure in polydioxanone is proportional to the sternal wire.

Keywords: Sternal wire, Polydioxanone, Sternum ultrasonography, Paediatrics

Introduction

Sternotomy was first introduced in 1997 by using experimental goats and human cadaver which then succeeded, then in 1957, performed sternotomy action on heart surgery patients¹². In the USA, more than 750,000 median sternotomy procedures are performed
Today, closure of the sternum bone that is often used by using stainless steel wire material (SS). The commonly used closure techniques are figure-of-8 and simple interrupted techniques. Biomechanical studies on cadavers and animals show the efficacy of several sternal closure techniques with different results 4–6. Currently, the use of synthetic polydioxanone absorbable suture after sternotomy becomes a routine procedure performed in many heart surgery centers, especially in cases of heart surgery in pediatric patients. Several studies have shown the use of polydioxanone (PDS) is effective in preventing sternal instability, assisting wound healing and is more suitable for pediatric patients 7,8. In addition, the PDS handling is easier to use, lowering the risk of bleeding at the stitching site and reducing postoperative pain 9–11.

The use of PDS in cardiovascular surgery was first performed on the sternum closure procedure for various heart operations such as correction of coarctation of the aorta, total correction Pulmonary vein drainage anomaly, arterial switches in Transposition of Great Arteries and systemic pulmonary shunts. Postoperative results obtained polydioxanone does not cause inflammatory and mediastinitis reactions, at least granulation tissue and is well-absorbed within the span of 2-6 months. The strength of fixation on the sternum is influenced by the type of fixation technique, the amount, strength and thickness of the wire used and the strength of the sternum itself 12,13.

The main goal to be achieved from the sternum closure technique is the achievement of sternal healing after heart surgery without any complications due to sternal healing failure. There are two important factors in bone healing process including mechanical stress and movement which can affect sternum technique stability in callus formation process, the formation or gap between the two sides of the split sternum and the fixation of movement between the sternal fragments 14,15.

The routine procedure performed in Dr.Soetomo General Hospital in sternal closure after childhood heart surgery is the most frequent use of continuous suture Polydioxanone yarn while the SSW of the figure of eight is rarely used. Evaluations to assess sternal healing include clinical evaluation of pain scale and physical examination scale assessing sternal stability using the scale 16. This evaluation was used in several studies of sternal stability assessment after adult heart surgery 17. But there has not been much study in patients after child’s heart surgery.

Based on, the study literature comparing the two uses of this material to a child’s heart surgical patient as well as evaluating the ratio of its cure. Until now, no research results have been found, therefore, this study intends to compare the effect of sternum closure technique on the patient’s heart surgical patients using Polydioxanone and Stainless Steel Wire threads on clinical stability and rate of healing which is evaluated clinically and Ultrasonography.

**Method**

This study used a double-blinded randomized clinical trial design in patients with cardiac surgery performed by the sternal closure. The study was conducted in the operating room of cardiac surgery, radiology ultrasonography examination room and polyclinic surgery of Toraks, Kardiak, and Vascular Dr.Soetomo General Hospital, Surabaya. The study was conducted from 2016 to 2017. The inclusion criteria included pediatric patients undergoing open and closed heart surgery with a 1-10-year-old sternotomy approach with 10-20 kg. Redo surgery, re-surgery due to postoperative hemorrhage and the patient dies or did not control during the observation period of the study was excluded.

The research procedure, covering the selection phase of the patient according to the inclusion criteria, then the research tool needed sternum closure technique using two types of material include Stainless steel wire and polydioxanone synthetic yarn. Furthermore, in the treatment stage, all patients undergoing standardized cardiac surgery procedures adhere to diagnose and prevailing protocols. The suturing process will use at least 3 SS wire fixations with the figure of -8 and 6 continuous stitching Polydioxanone for each sternum. Furthermore, the observational stage of the study, the patient was evaluated postoperatively with evaluation at the cardiac surgical polyclinic at 9th, 12th week. Sternum ultrasound examination with the transversal projection at three checkpoints (upper sternum, mid sternum, and lower sternum). Statistical data processing by using Mann Whitney Test for the non-parametric test with SPSS v23 program (SPSS, Inc., Chicago, IL.).
Results

The first group of sternal bone was fixed with sternal wire with the stitch of the figure of eight and the second group was fixed with PDS with simple continuous stitching. Patients were followed for postoperative physical and radiological examination at weeks 6, 9 and 12. The sternal closure images with PDS and sternal wire bias are seen in Figures 1 and 2.

The results of stability were obtained in the 6th-week evaluation results in patients using sternal wire, showed a higher degree of pain compared with PDS (p = 0.03) with sternal wire group pain characteristics in mild to moderate to severe degrees. While in the PDS group all patients felt a mild degree of pain. Evaluation at week 9 of patients using sternal wire still showed the higher degree of pain than PDS (p = 0.01) but with lower pain level characteristics. All patients with sternal wire experience mild pain up to moderate pain level. In the 12th week, both sternal wire and PDS were not found significant differences in pain (p = 1.000).

At the 6th-week ultrasound examination, the use of PDS showed a significant displacement compared to the use of sternal wire (p = 0.025). At 9th week ultrasound evaluation still showed significant displacement compared to sternal wire (p = 0.009) and at week 12 evaluation PDS still showed a significant displacement compared to sternal wire (p = 0.009) but no new displacement at other patients.

The results In the sixth week USG examination found a significant gap difference which obtained a wider gap in PDS in Manubrium (p = 0.021) while at mid and lowers there was no significant gap difference (p = 0.094 and p = 0.728). On the ultrasound examination also has not obtained any callus picture either in the PDS group or sternal wire.

In the 9th-week gap assessment, there was a significant difference with the wider gap of PDS in Manubrium sternum (p = 0.005) and on the sternum Corp (p = 0.001) while on the lower sternum there was no significant gap difference (p = 0.069). In the 12th week, there was a significant difference with the wider gap in the PDS group, i.e., in the Manubrium sternum (p = 0.001), the sternum Corp (p = 0.002) and the lower sternum (p = 0.029).

When compared to the number of gap additions at the 6th, 9th, and 12th-week checks, the gap size increased significantly from week 6 to 9 in the PDS group compared to the sternal wire group. The addition of a gap occurs at all points of the sternal examination. The addition of the gap at the 9th to the 12th week was not statistically significant but it appears that PDS has a wider gap addition range than the sternal wire.

In this 9th week, we have got callus picture on PDS group and sternal wire. The number of patients seen callus in the sternal wire group was more than the PDS, but not statistically significant compared to the PDS group (P = 0.602). In the 12th week of ultrasonography results, there was a callus profile of both the PDS and the sternal wire with more callus numbers obtained in sternal wire patients but not statistically significant (p = 0.333).

Table 1. Characteristics of the patients

<table>
<thead>
<tr>
<th></th>
<th>Sternal wire</th>
<th>PDS</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Male</td>
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<tr>
<td>Sex</td>
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<tr>
<td>Male</td>
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<td>Weight (kg) 10–20</td>
<td>Mean</td>
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<tr>
<td></td>
<td>12.43 ± 3.150</td>
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<tr>
<td>Age(y/o) 0–12</td>
<td>8.90 ± 1.560</td>
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### Table 2. Procedure operation

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<tr>
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<tr>
<td>BCPS</td>
<td>2</td>
<td>12.5</td>
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<tr>
<td>BT Shunt</td>
<td>2</td>
<td>12.5</td>
</tr>
<tr>
<td><em>corpus alienum</em></td>
<td>1</td>
<td>6.3</td>
</tr>
<tr>
<td>TOF Total Correction</td>
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<td>18.8</td>
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<tr>
<td>VSD Closure</td>
<td>4</td>
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<tr>
<td><strong>Total</strong></td>
<td>16</td>
<td>100.0</td>
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### Table 3. Stability check results

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<th>Week-9</th>
<th>Week-12</th>
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<td>Mild</td>
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<td>8</td>
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</tr>
<tr>
<td>Mod</td>
<td>5</td>
<td>3</td>
<td></td>
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<tr>
<td>Sev</td>
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<tr>
<td>p-price</td>
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<td>0.01</td>
<td>1.00</td>
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<td>7</td>
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<tr>
<td>Minimal</td>
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<td>2</td>
<td>1</td>
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<tr>
<td>Complete</td>
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<tr>
<td>Harga p</td>
<td>0.143</td>
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<tr>
<td>Displace</td>
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<td>p-price</td>
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### Table 4. Healing rate results

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<td>6</td>
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<tr>
<td></td>
<td></td>
<td>p-price</td>
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<td>0.602</td>
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<tr>
<td></td>
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<td>Manubrium (mm)</td>
<td>1.31 ± 0.125</td>
<td>1.47 ± 0.12</td>
<td>1.35 ± 0.16</td>
<td>1.75 ± 0.25</td>
<td>1.38 ± 0.20</td>
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<tr>
<td></td>
<td></td>
<td>P</td>
<td></td>
<td>0.021</td>
<td>0.005</td>
<td>0.001</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Corpus (mm)</td>
<td>1.13 ± 0.08</td>
<td>1.21 ± 0.09</td>
<td>1.14 ± 0.07</td>
<td>1.38 ± 0.13</td>
<td>1.18 ± 0.06</td>
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<tr>
<td></td>
<td></td>
<td>P</td>
<td></td>
<td>0.094</td>
<td>0.001</td>
<td>0.019</td>
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<tr>
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<td></td>
<td>Lower sternum (mm)</td>
<td>1.16 ± 0.05</td>
<td>1.16 ± 0.10</td>
<td>1.16 ± 0.05</td>
<td>1.22 ± 0.07</td>
<td>1.18 ± 0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
<td></td>
<td>0.728</td>
<td>0.105</td>
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### Table 5. Average of gap increase

<table>
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<th>Variable (sternal wire vs PDS)</th>
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<th>Week 9 to 12</th>
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</table>

### Discussion

The stability and healing of the sternum in this study was stability and clinical and radiological healing evaluation at 6th week, 9th week and 12th week. The sternal stability was evaluated based on the degree of pain on clinical examination as well as the physical examination to assess sternal stability and then evaluate the presence or absence of displacement by examining ultrasonography. While the healing of the sternum was assessed through ultrasonography examination based on the evaluation of the gap on the sternum as well as the callus picture on the sternal fragment.

The stability and healing process of the sternum is influenced by the combination of daily physiological movements such as breathing, coughing and moving that create a force on the sternum. The force was the
result of a combination of transverse shear, longitudinal shear, and lateral distraction movements. In this study at weeks 6, 9 and 12 seen in ultrasound there was a significant displacement in the PDS group. This study was in accordance with the study wherein the Sternal wire group with strong material properties, proved to have a much better rigidity compared to PDS and this was indicated by a stable symmetry of the sternum.

At week 6 there was a wider gap in the Manubrium and Corpus Sternum groups of PDS than in Sternal wire. The gap differences remained significantly larger at the 9th and 12th weeks. This difference is due to the fact that the PDS uses absorbable material which will certainly be degraded over a period of time. Based on the PDS profile of ethicon® the power of the PDS at week 2 will decrease to 70% and will decrease gradually to 25% at 6th week.

At the end of the 12th week of study, the PDS gap compared to the sternal wire was 19.25 ± 1.83 vs 13.75 ± 1.58. This shows the evaluation of PDS usage by ultrasonography is seen widening and addition of bigger gap than sternal wire. Although the use of Sternal wire proves to be more confined in fixing the Sternum, PDS threads were also able to maintain the gap of Sternum with a distance below 2 mm can trigger the running of the healing process (fibrous tissue healing).

**Conclusion**

Sternal wire causes higher pain than PDS. Clinically, sternal wire stability was proportional to PDS. Radiologically, the stability of sternal wire was better than PDS. PDS produces a wider sternal gap than a sternal wire. The rate of sternal healing in the PDS was proportional to the sternal wire.

**Conflict of Interest:** There is no conflict of interest

**Source of Funding:** This study is self-funded

**Ethical Clearance:** This study was approved by Ethical Comission of Health Research Faculty of Medicine University of Airlangga.

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Discrimination the Gender in the Criminal Evidence at Crime Scene

Qutaiba S. H. AL-Ameedy1, Noor Salman Kadhim Al-Khafaji2

1Biologist at Babylon Health Directorate, Ministry of Health,
2Assistant professor at Biology Department, College science-University of Babylon, Iraq

Abstract

Background: An ideal method for gender identification would be accurate, simple, and cheap, enabling its use in most laboratories. In addition, it should also be able as much as possible to be used for all animals as well as tissues and/or cells. For chromosomal analysis, we need viable cells that are able to divide, and if this is not possible, these methods cannot be used. On the other hand, genetic methods are reliable and do not need living cells, and it is easy to obtain DNA for these studies even in very ancient and nonviable tissues. These methods are therefore the most accepted ones. The current study aimed to determine the gender of a given DNA samples by designed valuable specific-PCR tool.

Methodology: Thirty eight (nineteen from each male and female) human blood samples were collected using EDTA-Na2 tubes for direct DNA extraction after taken the agreement of the volunteers to give the blood sample via expert. The blood sample mixed well by rotation of EDTA tube. Two sets of species specific primer pairs targeting Homo sapiens (AMLE X and AMLE Y) genes were designed and checked. PCR and sequencing were performed and sequences were analyzed and register in GenBank. Results: The results revealed that the amplicon of AMLE X gene of Homo sapiens were 1164bp and AMLE Y were 744bp

Conclusion: The current study conclude that, validity and accuracy of designed 12S rRNA species specific primer pairs for human and nonhuman animal blood typing as a forensic tool and there is no intraspecies cross amplification.

Keywords: Homo sapiens, AMLE X and AMLE Y.

Introduction

Analysis of the gender chromosomes is important in the determination of gender and instantly excludes 50% of the population. The human gene usually analyzed to determine gender is the Amelogenin (AMEL) locus, after its amplification using PCR DNA fragments of different lengths can be generated. Amelogenin is involved in the formation of enamel, which is the hard, white material that forms the protective outer layer of each tooth[1]. Enamel is composed mainly of mineral-containing crystals. These microscopic crystals are arranged in bundles that give enamel its strength and durability[2]. Studies suggest that lesser amounts of amelogenin may also be present in tissues other than developing tooth enamel. For example, amelogenin has been found in certain bone, bone marrow, and brain cells. The function of amelogenin in these tissues is unknown. One copy of the amelogenin gene is located on each of the sex chromosomes (the X and Y chromosomes)[3]. The sequence on the X chromosome is shorter by 6 bp compared to the allele on the Y chromosome (male gender)[4]. Polymerase Chain Reaction (PCR) analysis that target regions of Amelogenin gene have become the method of choice for gender determination of biological samples[5]. The aim of this study is to determine the gender of a given DNA samples by designed valuable specific-PCR tool.

Methodology

Sampling:

Thirty eight (nineteen from each male and female) human blood samples were collected using EDTA-Na2 tubes for direct DNA extraction after taken the agreement of the volunteers to give the blood sample via expert. The blood sample mixed well by rotation of EDTA[6].
DNA Extraction Polymerase Chain Reaction:

G-spinTM Total DNA Extraction Kit (was used to extract DNA from blood according to the manufacturer’s protocol instructions (Intronbio/Korea). All extracted DNA samples were submitted for Nanodrop to ensure adequate purity and concentration required for PCR. Setting done by selection dsDNA measuring mode for 2 μl of sample. Blanking were performed using 2 μl microliter of elution buffer and then measuring the DNA samples concentration and purity[7].

The sequence of Homosapiens (AMLE X, AMLE Y) were taken from NCBI data base (AMLE X Sequence ID: AY040206.1; AMLE Y Sequence ID: NG_008011.1) were used to design primer pairs using online Primer 3 software [8,9]. The specific primer pairs: AMLEX-F: CTGGAGGGTCTTGCTGAAG, AMLEX –R: GATGTACCAAAGGGGTTGGG( 1164 bp) ; AMLEY-F: CCAGGACAGCTAGGTTCAAGTT, AMLEY –R: CACCTCCATAAAATAGCTTTGTC (744 bp). The specificity of designed primer pairs were checked using online NCBI/primer blast [9,10]. The PCR conditions were calculated using online Protocol Optimize writer software. The conditions were illustrated in table (1).

Sequence Analysis

Trimming of sequences were perform by FinchTV and then submitted to NCBI-BLASTN to see the identity of sequences with reference sequences within NCBI data base.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Conditions</th>
<th>References</th>
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</thead>
<tbody>
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<td>AMLEX –F</td>
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<td>This study</td>
</tr>
<tr>
<td>AMLEX -R</td>
<td>30sec.</td>
<td></td>
</tr>
<tr>
<td>AMLEX –F</td>
<td>30sec.</td>
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</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>AMLEY –R</td>
<td>2min.</td>
<td></td>
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</table>

Results and Discussion

The results of PCR reveal that, firstly the primer pairs were specific and non-specific products not appear for all samples. The amplification of male and female Homo sapiens DNA with AMLEY primer pairs gave positive and negative results respectively while The amplification of male and female Homo sapiens DNA with AMLEX primer pairs gave positive results for all samples and this a primary evidence for primer pairs specificity The amplicon of AMLEY gene of Homo sapiens 744bp (Figure 1: A). The amplicon of AMLE X gene of Homosapiens were 1164 bp (Figure 1:B).
Figure 1: Agarose gel electrophoresis at 72 volt for 60 minutes 1.5% for A: 744bp amplicon of Homo sapiens AMLE Y gene; B: 1164bp amplicon of Homosapiens AMLE X gene. M represent 100bp DNA ladder, lane 1-19 represent male samples. lane 20-38 represent female samples.

The secondary and confirmatory assay for specificity of primer pairs used in study is sequences of PCR products. four amplicons from each were sent to South Korea for sequencing using Sanger technique (Macrogen/Korea). The retrieved sequences firstly must be trimmed to remove unwanted sequences before submitting them for BLASTN. The trimming performed by Bioedit to get the finally processed sequences. Abbreviation of AMLE Y sequences were used as IraqiAMELY-1 to IraqiAMELY-4. Same abbreviation will used as isolates name when submitting to GenBank. The identity percentage were illustrated in Table (2). The high percentage of identity of sequences confirm the specificity and validity of specific primer pairs used in current study.

Table (2): Identity of blasted samples (IraqiAMELY-1 to IraqiAMELY-4) with reference sequences of highest identity percentage

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Sequence ID</th>
<th>Identities</th>
<th>Gaps</th>
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<tr>
<td>IraqAMELY-1</td>
<td>NG_008011.1</td>
<td>453/453(100%)</td>
<td>0/453(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IraqAMELY-2</td>
<td>NG_008011.1</td>
<td>446/446(100%)</td>
<td>0/446(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IraqAMELY-3</td>
<td>NG_008011.1</td>
<td>450/450(100%)</td>
<td>0/450(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IraqAMELY-4</td>
<td>NG_008011.1</td>
<td>446/446(100%)</td>
<td>0/446(0%)</td>
<td>Plus/Plus</td>
</tr>
</tbody>
</table>

Concern the abbreviation of AMLE X sequences be IraqiAMELX-1 to IraqiAMELX-4. The identity percentage results were illustrated in Table (3). The high percentage of identity of sequences confirm the specificity and validity of specific primer pairs used in current study.
Table (3): Identity of blasted samples (IraqiAMELX-1 to IraqiAMELX-4) with reference sequences of highest identity percentage

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Sequence ID</th>
<th>Identities</th>
<th>Gaps</th>
<th>Strand</th>
</tr>
</thead>
<tbody>
<tr>
<td>IraqAMELX-1</td>
<td>NG_012040.1</td>
<td>391/391(100%)</td>
<td>0/391(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IraqAMELX-2</td>
<td>NG_012040.1</td>
<td>409/409(100%)</td>
<td>0/409(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IraqAMELX-3</td>
<td>NG_012040.1</td>
<td>733/733(100%)</td>
<td>0/733(0%)</td>
<td>Plus/Plus</td>
</tr>
<tr>
<td>IraqAMELX-4</td>
<td>NG_008011.1</td>
<td>630/630(100%)</td>
<td>0/630(0%)</td>
<td>Plus/Plus</td>
</tr>
</tbody>
</table>

The results showed no cross-reactivity of designed primer pairs and the PCR assay based on the designed primer pairs will be simple, fast, sensitive, specific, and cost-effective.

Genetic sex determination methods are not related to subjective physical examination, are accurate, require small samples, and do not necessitate the evaluation of a specific tissue, and any organ can be used. Their applicability depends on the specific methods. Successful assays are simple, need small amount of tissue, and are accurate during the entire pregnancy. Measuring the activity of X chromosome linked enzymes or RNA-based PCRs is complicated by the presence of some gene products only at certain developmental stages\(^\text{[1]}\). However, this problem is not present when the test is based on DNA. The amelogenin gene which is found on both X and Y chromosomes is in common use for sex discrimination in forensic medicine. A 6 bp deletion in intron 1 on chromosome X compared to the Y chromosome can be detected by using a pair of PCR primers. It can be used in various tissues including long-lasting remnant tissues like dental pulp\(^\text{[12]}\).

**Conclusion**

The current study concludes Sensitivity, specificity of the designed specific primer pairs; Applicability of the designed primer pairs in forensics to investigate blood sample or evidence belonging for detection of gender.

**Ethical Clearance:** The project plan displayed on the scientific committee and scientific ethical committee of the department of Biology-college of science at university of Babylon and get approval.

**Source of Funding:** There is no funding source and it is completely covered by authors

**Conflict of Interest:** There is no conflict of interest.

**References**


5. Mitchell RJ, Kreskas M, Baxter E, Buffalino L, Van Oorschot RA. An investigation of...


Effect of Workload and Breastfeeding Motivation of Working Mothers

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Abstract

Background: The practice of exclusive breastfeeding in Indonesia still falls short of expectations due to working mothers’ struggle with managing both working outside and household chores. This workload results in mothers experiencing physical and emotional exhaustion and impacts on their motivation to practice exclusive breastfeeding, hence exclusive breastfeeding failure.

Objective: To shed light on the relationship between workload and exclusive breastfeeding motivation of working mothers in the working area of the Public Health Center of Bergas District, Semarang Regency.

Method: This is a cross-sectional study with a sample of working mothers in the working area of the Public Health Center of Bergas District, Semarang Regency, with babies aged 0–6 months. The sample of 32 respondents was recruited by purposive sampling technique. The data collected were analyzed by Spearman’s rho analysis. The instruments employed in this research were the modified versions of questionnaires from existing literature.

Results: The workload of nearly all of the sample (31 respondents, 96.8%) was categorized into Level 2. Besides, the vast majority of the breastfeeding, working mothers in Bergas District (30 people, 93.7%) had strong exclusive breastfeeding motivation. Last but hardly the least, a ρ-value of 0.801 (> 0.05) was obtained, suggesting no relationship between workload and exclusive breastfeeding motivation.

Conclusion: Workload bore no association with the exclusive breastfeeding motivation of working mothers. Despite the high workload, if the reinforcement system was high, individual motivation would generally remain high.

Keywords: workload, exclusive breastfeeding motivation, working mothers

Introduction

Exclusive breastmilk is human milk given to infants from birth until six months of age without the accompaniment of food or drink. Exclusive breastfeeding is followed by supplementation until the baby reaches two years of age 1. In Indonesia, the exclusive breastfeeding practice fails to live up to expectations. Exclusive breastfeeding practice failure is still prevalent, and the rate is higher in mothers working at institutional offices, government- or private-run, than in those working from home. In the case of those engaged in office work, time and rule inflexibility often sets a barrier to spending time together with their babies and takes a toll on their breastfeeding intensity 2. The government through Regulation of the Minister of Health of the Republic of Indonesia of 2014 set the exclusive breastfeeding scope target at 80%, but in parts of its territory, the percentage barely could touch as high as 50% 3.

Exclusive breastfeeding support, especially for working mothers, is manifested through global and national policies. One of such policies is Government Regulation No. 33 of 2012 on Exclusive Breastfeeding.
Another is set out in Article 2 of Joint Ministerial Regulation of the Minister of Women’s Empowerment, the Minister of Manpower and Transmigration, and the Minister of Health No. 48/MEN-PP/XII/2008/PER27/MEN/XII/2008 on Breastfeeding Promotion at Workplace. Meanwhile, global support came from UNICEF through a statement in 2013 that exclusive breastfeeding for a minimum of six months makes the lowest-cost life-saver for children.

In addition to the demand from their full-time work, mothers are also faced with household chores, including nursing their babies, after work. This phenomenon is referred to as second shift. The implications include mothers’ inability to complete their household tasks maximally due to physical and emotional fatigue. It is assumed then that this second-shift-driven physical and emotional exhaustion will influence mothers’ exclusive breastfeeding motivation.

In light of the abovementioned, this research intended to observe whether relation does exist between workload and exclusive breastfeeding motivation of working mothers. The aim of this research was to identify mothers’ workload and motivation to engage in breastfeeding practice.

Materials and method

This research is a quantitative study with a cross-sectional design, in which the relationship of independent to dependent variables was measured at one time with no follow-up.

Population and sample

The sample enrolled comprised working mothers in the working area of the Public Health Center of Bergas District, Semarang Regency. The inclusion criteria used were that respondents worked outside, had baby 0–6 months old, resided in the working area of the Public Health Center of Bergas District, and were breastfeeding. Meanwhile, the exclusion criteria were that respondents were suffering from mental disorder, were unemployed or worked at home (operating online business or operating local shop or booth at home), and resided beyond the working area of the Public Health Center of Bergas District, Semarang Regency. A sample of 32 respondents was derived from a population of 47 working mothers. This number had surpassed the minimum number of subjects for correlational studies of 30. The sampling technique employed was purposive sampling technique.

Research variables

The independent variable in the present study was workload, whereas the dependent was working mothers’ exclusive breastfeeding motivation.

Research instruments

The instruments employed in this study were questionnaires modified from existing literature. For the variable workload, the instrument adopted was the Fatigue Likelihood Scoring questionnaire by Transport Canada, consisting of five questions regarding total working hours per week, maximum working duration per shift, minimum interval between two shifts, maximum night shift duration per week, and off-day frequency. The scores generated would point to the final position in the five-level fatigue scale below.

### Table 1 Fatigue Likelihood Scoring Blueprint

<table>
<thead>
<tr>
<th>Scores</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total working hours per week</td>
<td>≤ 36 h</td>
<td>36.1–43.9</td>
<td>44–47.9</td>
<td>48–54.9</td>
<td>55+</td>
</tr>
<tr>
<td>Maximum shift duration per day</td>
<td>≤ 8 h</td>
<td>8.1–9.9</td>
<td>10–11.9</td>
<td>12–13.9</td>
<td>14+</td>
</tr>
<tr>
<td>Minimum rest time before setting out for work the next day</td>
<td>≥ 16 h</td>
<td>15.9–13</td>
<td>12.9–10</td>
<td>9.9–8</td>
<td>≤ 8</td>
</tr>
</tbody>
</table>
As for the variable exclusive breastfeeding motivation, the measurement was conducted by the Breastfeeding Motivation Instructional Measurement Scale (BMIMS) questionnaire, previously subjected to expert-based content validity test and then selected in this research based on the measurement parameters. The lowest score obtained was 12, and the highest 48. The scores obtained were then converted into percentages (%), according to which, as stated by Suadana et al. (2018) that motivation was categorized, with scores between 67–100% being strong, 34–66% medium, and 0–33% weak.

It was identified from this research that nearly all respondents scored 9 to 18 for the Fatigue Likelihood Scoring (FLS). Based on the response analysis, the workload fell into such a range owing to the fact that a great majority of the respondents worked a total of 55 hours every week (in the scoring system belonging to the score 8). In other words, they spent 10 to 11 hours a day (score 2) 5–6 days a week working, with only 8–9 hours a day for rest (score 4). Besides, they were given off-day 1–2 times weekly (score 1). When everything was added up, the final score fell to the 9–18 range. Based on the Fatigue Likelihood Scoring guide, the score range denoted Level 3 fatigue, which belonged to the yellow area of the instrument 7.

Validity test

Validity testing was run by consumer validity, that is, by distributing questionnaire to 15 respondents who were enrolled by accidental sampling technique. The respondents were chosen for sharing similarities in characteristics with the sample. The correlation coefficient from the validity testing was compared to the coefficient in the Pearson’s product moment correlation table with 5% margin of error (0.05).

Reliability test

The reliability test run against the questionnaire used Cronbach’s alpha formula. The counted $r$ alpha was compared to the table $r$ alpha. If the counted $r$ alpha was greater than the table $r$ alpha, the question item would be declared reliable and fit to be used in the research, but if the counted $r$ alpha was less than the table $r$ alpha, the question item would not be used in the research 9.

Data analysis

This research employed a bivariate analysis of Spearman’s rank correlation. Spearman’s rank correlation, or Spearman’s rho, is a test used to measure the correlation between two variables with ordinal data or with interval- or ratio-turned-ordinal data and to look at the fitness of the variables tested into equivalent group 10.

Results

Table 2 below presents the respondents’ characteristics by age, education, and occupation. The majority of the respondents were factory workers aged 31 years on average (productive age). They mostly had high school or the equivalent as their latest formal education.
Table 2 General Data

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20–25</td>
<td>4</td>
<td>12.5%</td>
</tr>
<tr>
<td>26–30</td>
<td>12</td>
<td>37.5%</td>
</tr>
<tr>
<td>31–35</td>
<td>11</td>
<td>34.3%</td>
</tr>
<tr>
<td>36–40</td>
<td>4</td>
<td>12.5%</td>
</tr>
<tr>
<td>41–45</td>
<td>1</td>
<td>3.1%</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elementary school/equivalent</td>
<td>3</td>
<td>9.3%</td>
</tr>
<tr>
<td>Junior high school/equivalent</td>
<td>8</td>
<td>25%</td>
</tr>
<tr>
<td>Senior high school/equivalent</td>
<td>20</td>
<td>62.5%</td>
</tr>
<tr>
<td>Diploma</td>
<td>1</td>
<td>3.1%</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Private employee</td>
<td>1</td>
<td>3.1%</td>
</tr>
<tr>
<td>Cleaning service staff</td>
<td>1</td>
<td>3.1%</td>
</tr>
<tr>
<td>Factory worker</td>
<td>30</td>
<td>93.7%</td>
</tr>
</tbody>
</table>

The specific data presented in Table 3 were collected from the questionnaire responses. The data were comprised of six components: distribution of exclusive breastfeeding information; source of exclusive breastfeeding information; lactation facility availability at workplace; exclusive breastfeeding practice; exclusive breastfeeding motivation; and workload.

Table 3 Special Data from Working Mothers’ Questionnaire Responses

<table>
<thead>
<tr>
<th>General Data</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exclusive breastfeeding information</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Received</td>
<td>31</td>
<td>96.8%</td>
</tr>
<tr>
<td>Not received</td>
<td>1</td>
<td>3.1%</td>
</tr>
</tbody>
</table>
Table 3 provides a picture that the respondents mostly had strong exclusive breastfeeding motivation. It was supported by their knowledge on exclusive breastfeeding virtually all of them possessed, lactation facility availability at the workplaces at which most of them worked, and counselling from a range of parties, such as health professionals, mass, and electronic media. Despite all of the previously stated, only half of the total respondents did practice exclusive breastfeeding.

Spearman’s rho was used to analyze the relationship between workload and working mothers’ exclusive breastfeeding motivation. The results are as presented in Table 4, where it can be seen that the $p$-value was $0.801 (> 0.05)$.

<table>
<thead>
<tr>
<th>Source of information</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Health professional</td>
<td>15</td>
<td>48.3%</td>
</tr>
<tr>
<td>Electronic media</td>
<td>1</td>
<td>3.2%</td>
</tr>
<tr>
<td>Mass media</td>
<td>1</td>
<td>3.2%</td>
</tr>
<tr>
<td>Mass media and electronic media</td>
<td>3</td>
<td>9.6%</td>
</tr>
<tr>
<td>Health professional, PKK* gathering, workplace</td>
<td>1</td>
<td>3.2%</td>
</tr>
<tr>
<td>Health professional and PKK* gathering</td>
<td>2</td>
<td>6.4%</td>
</tr>
<tr>
<td>Health professional and mass media</td>
<td>2</td>
<td>6.4%</td>
</tr>
<tr>
<td>Health professional, electronic media, and PKK* gathering</td>
<td>4</td>
<td>12.9%</td>
</tr>
<tr>
<td>Pregnancy club</td>
<td>1</td>
<td>3.2%</td>
</tr>
<tr>
<td>Workplace</td>
<td>1</td>
<td>3.2%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lactation facility at workplace</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Available</td>
<td>30</td>
<td>93.7%</td>
</tr>
<tr>
<td>Unavailable</td>
<td>2</td>
<td>6.25%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Exclusive breastfeeding practice</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Engaged</td>
<td>15</td>
<td>46.8%</td>
</tr>
<tr>
<td>Unengaged</td>
<td>17</td>
<td>53.1%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Workload</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 2</td>
<td>1</td>
<td>3.1%</td>
</tr>
<tr>
<td>Level 3</td>
<td>31</td>
<td>96.8%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Exclusive breastfeeding motivation</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Strong</td>
<td>30</td>
<td>93.7%</td>
</tr>
<tr>
<td>Medium</td>
<td>2</td>
<td>6.25%</td>
</tr>
</tbody>
</table>

*PKK: Family Welfare Guidance Program
Table 4 Cross-tabulation of Workload and Working Mothers’ Exclusive Breastfeeding Motivation

<table>
<thead>
<tr>
<th>Workload</th>
<th>Exclusive Breastfeeding Motivation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strong</td>
</tr>
<tr>
<td>Level 2</td>
<td>1</td>
</tr>
<tr>
<td>Level 3</td>
<td>29</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
</tr>
</tbody>
</table>

Spearman’s rho ($\rho$) = 0.801

Correlation coefficient = 0.046

Discussion

This research aimed to analyze the correlation between workload and the exclusive breastfeeding motivation of working mothers in the working area of the Public Health Center of Bergas District, Semarang Regency. As indicated by the data above, the factors included in the respondents’ workload were working hours, rest time, off-day frequency, and age, so there was an agreement between the theory and the fact 11. It was found that there was no or negligible correlation between workload and the exclusive breastfeeding motivation of working mothers as shown by the correlation coefficient of 0.046 (0.00–0.20) in Table 4. It is known that the higher the workload, the higher the level of fatigue 12. Workload has long been measured by fatigue indicator as fatigue is resulted from unreasonable workload.

The factors influencing strong motivation of their respondents, as were in this research, including possession of adequate information from health personnel, mass media, electronic media, workplace, or regular events in residential neighborhood and availability of lactation facility at workplace 13. An additional factor was the fact that most of the respondents were in their adulthood. Institution-initiated programs, activities, and campaigns pertaining to exclusive breastfeeding promotion in this context could also influence the exclusive breastfeeding motivation of working mothers 4.

The last aspect was the motivation triggered by moving information from media to do something. In this instance, bottle-feeding as well as exclusive breastfeeding promotions affected one’s decision to exclusively breastfeed. It was as found in the present study that the respondents had gained information regarding the importance of exclusive breastfeeding in the first six months in baby’s life through media, either print or electronic 14. Motivation is aroused by the desire to achieve a goal, hope, and belief that the action taken will lead to a reward. Hence, if the desire, hope, and belief are high, an individual’s motivation will also be high, and vice versa 15.

Conclusion

The results of this research indicated that workload was not related to exclusive breastfeeding motivation, although the respondents’ workload belonged to Level 3 category with an FLS score within the 9–18 score range. The strong motivation of most of the working mothers in this research was driven by strong social support from the environment. The factors involved in the respondents’ reinforcement system positively impacted their belief, desire, and hope.

Statement of Informed Consent

Upon acceptance, all copyright ownership for the above-named paper is transferred to Indian Journal of Forensic Medicine & Toxicology. We, the undersigned authors of this paper, have contributed to (1) data design, analysis, or interpretation, and (2) writing or critiquing drafts of the manuscript, and we approve the submitted
version of the manuscript. We declare that this paper has not been published elsewhere in whole or in part in any language (except in abstract form). We are jointly responsible for the release of any or all of the material contained within the paper. We declare that we obtained patient consent-to-disclose forms for any figures of identifiable patients and edited out any names mentioned in the recording.

The consent form indicated specific use (reproduction in the medical literature in print and online, with the understanding that patients and the public will have access) of the patient’s medical information and any images and contained the patient’s signature or that of a legal guardian. We also declare that research involving human subjects was performed in compliance with the principles of the Declaration of Helsinki (2008), and that involving animals was approved by the applicable institutional ethics committee or review board and conformed with generally accepted guidelines governing such work. We further attest that we have herein disclosed any and all financial and other relationships that could be construed as conflicts of interest, and that all sources of financial support for this study are disclosed in the manuscript.

**Ethical Clearance**

This research process is done that was accordant with the ethical research principle based on the regulation of research ethic committee. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, nonmaleficence, and justice.

**Conflict of Interest:** We declare that there is no report or issue about conflict of interest related with this paper.

**Source of Funding:** All funding is done by the authors with no external support.

**References**


Effectiveness of an Educational Program on Nurses- midwives’ Practices about Pain Management during Labor in Baghdad Maternity Hospitals

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1Instructor, Maternal and Neonate Nursing Department, College of Nursing/ University of Baghdad, 2Professor, Maternal and Neonate Nursing Department, College of Nursing, University of Baghdad, 3Assist. Prof. Dr. College of Medicine, Al-Iraqia University

Abstract

Objectives: To assess nurses-midwives’ practices about pain management during labor before and after implementation of educational program.

Methodology: A quasi-experimental design has been conducted during the period of (27th February 2019 through 2nd June 2019) on non-probability sample (purposive) consists of (44 Nurses/midwives’) who are work in delivery room, the sample was exposed to pretest, educational program, posttest. The study was conducted in the Baghdad Maternity Hospitals. Questionnaire has been used as a tool of data collection. Data were analyzed through the application of descriptive and inferential statistical data analysis approach through the use of (SSPS) version 22.0 and Excel system.

Results: All observations (three observations) in posttest period in the items (changing position, deep breathing, and religious and spiritual idea) appear high and moderate mean scores and relative sufficiency in the three observations after the implementation of education program for nurses- midwives’ regarding to the management taken by the midwife to reducing labor pain of pregnant women in delivery room. While other management not implemented by the nurses- midwives’ due to the lack in the resources or supplementation in delivery rooms, or due to the routine of the hospitals.

Conclusions: The study concluded that the educational program can be considered as an effective mean for improvement of the nurses-midwives’ practices about the importance pain management.

Recommendations: The study recommended to training primary health care nurses in improved pain management is important part of multi-faced approach towards improving and helping women’s to reduce pain during labor.

Keywords: Educational Program, Nurses- midwives’ Practices, Pain Management, Labor.

Introduction

Labor pain is one of the most intense pains of women. This type of pain is very common and is an inevitable part of the childbirth process. For some reasons, labor pain and its experience is quite different from other types of pains. This pain is not a symptom of tissue injury, and can spontaneously be limited and controlled. This pain gradually gets intense and eventually leads to a desirable event which is the childbirth (1)

Pain experienced during childbirth is a complex, multidimensional and subjective phenomenon that is of great concern to both the expectant mother and the maternity healthcare professional. Although the experience of pain is inherent in the childbearing process, unrelieved labor pain can result in negative consequences for the expectant mother, her family, healthcare providers and healthcare systems at large. Apart from maternal consequences such as heightened stress, fear, depression, confusion, hypertension, hyperglycemia, and constipation, unresolved labor
pain can also compromise placental perfusion leading to asphyxia, late decelerations and its resultant fetal distress. These create feelings of guilt and helplessness for the woman’s family as well as a lack of confidence in the abilities of healthcare providers and systems in general(2).

Methodology

A quasi-experimental design has been conducted during the period of (27th February 2019 through 2nd June 2019) on non-probability sample (purposive) consists of (44 Nurses/midwives’) who are work in delivery room, the sample was exposed to pretest, educational program, posttest. The study was conducted in three Directories, (Baghdad Teaching Hospital) at medical city health directorate, (Al-Elwia Maternity Teaching Hospital, Ibn Al-Zahra for Maternity and Pediatric Hospital, Fatima Al-Karckh Maternity Hospital and AL - Yarmouk Teaching Hospital - Maternity Department) at AL-Russafa/Health Directorate, and (Al -Karckh Maternity Hospital and AL - Yarmouk Teaching Hospital - Maternity Department) at Akarkh/Health Directorate. Questionnaire has been used as a tool of data collection to fulfill with objective of the study to assess nurses-midwives’ practices about pain management during labor before and after implementation of educational program and to determine the effectiveness of educational program on nurses-midwives’ practices about pain management during labor in Baghdad Maternity Hospitals. Data were analyzed through the application of descriptive and inferential statistical data analysis approach through the use of (SSPS) version 22.0 and Excel system.

Results

Table (1): Distribution of Socio-demographic Characteristics for Nurses-Midwives’:

<table>
<thead>
<tr>
<th>Socio-demographic Characteristics</th>
<th>Frequency</th>
<th>percent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-24</td>
<td>11</td>
<td>25.0</td>
</tr>
<tr>
<td>25-29</td>
<td>6</td>
<td>13.6</td>
</tr>
<tr>
<td>30-34</td>
<td>7</td>
<td>15.9</td>
</tr>
<tr>
<td>35-39</td>
<td>7</td>
<td>15.9</td>
</tr>
<tr>
<td>40-44</td>
<td>3</td>
<td>6.8</td>
</tr>
<tr>
<td>45-49</td>
<td>8</td>
<td>18.2</td>
</tr>
<tr>
<td>50-54</td>
<td>2</td>
<td>4.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>44</td>
<td>100.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Social Status</strong></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
<td>12</td>
<td>27.3</td>
</tr>
<tr>
<td>Married</td>
<td>30</td>
<td>68.2</td>
</tr>
<tr>
<td>Widowed</td>
<td>2</td>
<td>4.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>44</td>
<td>100.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Educational Level</strong></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Preparitory Nursing</td>
<td>3</td>
<td>6.8</td>
</tr>
<tr>
<td>Preparatory Midwifery</td>
<td>31</td>
<td>70.5</td>
</tr>
<tr>
<td>Midwifery Institute</td>
<td>10</td>
<td>22.7</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>44</td>
<td>100.0</td>
</tr>
</tbody>
</table>

\[ \bar{x} \pm SD \]

\[ \bar{x} : \text{mean, SD: Standard Deviation} \]
Table (1) shows that the highest percentage (25%) of the nurses-midwives’ are (20-24) years with mean and standard deviation (SD) (33.84 ± 9.4); (68.2%) were married; (70.5%) preparatory midwifery graduates.

Table (2): Management Taken by Nurses –midwives’ to Reduce Pain during Labor in Delivery Room in Pre and Post Observations.

<table>
<thead>
<tr>
<th>No.</th>
<th>Practical Items</th>
<th>Pre Observation</th>
<th>Post Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Frequency</td>
<td>MS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Always</td>
<td>Some times</td>
</tr>
<tr>
<td>1</td>
<td>Massage</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Changing position</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Therapeutic Touch</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>Deep breathing</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>5</td>
<td>Concentration and reflection</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>Essential oils</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>Warm and cold compresses</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>Religious and spiritual</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

F: Frequency, %: Percentage, MS.: Mean of Scores(weighted mean); Sd: Standard Deviation, RII.: Relative Importance Index , Ass.: Assessment, L: Low: (0.33–0.55), M: Moderate:(0.56–0.78), H: High: (0.79 – 1.00).

Table (2) results show that there are low mean scores and relative sufficiency in pretest period in all items. While there are high mean scores and relative sufficiency in posttest period and significant correlations in (changing position, deep breathing, and religious and spiritual idea), after the implementation of education program for nurse-midwives regarding to the management taken by the nurse to reducing labor pain.
Table (3): Nurse-midwife practices to reduce labor pains in the delivery room (clinical practice).

<table>
<thead>
<tr>
<th>First Observation</th>
<th>Second Observation</th>
<th>Third Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Frequency</td>
</tr>
<tr>
<td>1. The nurse-midwife massages women...</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. The nurse-midwife massages woman...</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. The nurse-midwife massages woman...</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. The nurse-midwife massages woman...</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. The nurse-midwife massages woman...</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. The nurse-midwife massages woman...</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. The nurse-midwife massages woman...</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. The nurse-midwife massages woman...</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Legend:
- MS: Always
- SD: Sometimes
- RS: Never

Frequency:
- Never
- Sometimes
- Always
F: Frequency, %: Percentage, MS.: Mean of Scores (weighted mean); Sd: Standard Deviation, RII.: Relative Importance Index, Ass.: Assessment, L: Low: (0.33-0.55), M: Moderate: (0.56-0.78), H: High: (0.79 – 1.00).

Table (3) results show that there are high and moderate mean scores and relative sufficiency in the first observations in posttest period practices (2, A, B, & K); (4, C); and (8) after the implementation of education program for nurse-midwives practices taken to reducing labor pain of pregnant women in delivery room. In the second observation there are high mean scores and relative sufficiency in practices (2, & K); (4); and (8). In the third observation there are high and moderate mean scores and relative sufficiency in practices (2, & K); (4, C); and (8).

**Discussion**

Socio-demographic Characteristics of the Study Sample

Table (1) shows that the highest percentages (25%) for study sample at age group (20-24) years with mean and (SD) (33.84 ± 9.4).

These findings are agreement with cross sectional study design that assess practice of Labor pain management methods and associated factors among skilled attendants in all Tigray region general hospitals. And found that out of the total of (233) skilled attendants were included in the study, making a response rate of 100%. The mean age of the respondents was (30.2 ± 6.6), of them 152(65.3%) were in the age group of (20-29), 56(24%) were in age group (30-39) and 25(10.7%) were in age group (≥ 40) (3). Conversely, the study conducted in two medical institutions providing health care services in Moi Teaching and Referral Hospital (MTRH) and Jaramogi Oginga Odinga Teaching and Referral Hospital, Kenya. The study found that the highest percentage (48.1 %) of health care providers were in the age group of (25-34) years (4). Table (1) shows that the highest percentages (68.2%) were married. Married nurses-midwives have more perception of the labor pain than an unmarried woman, because the first woman may have experienced pregnancy and labor and felt what pain a woman feels during labor. Table (1) shows that the highest percentages (70.5%) were preparatory graduates. These findings are disagreement with a quantitative design was used to examine differences in knowledge of pain assessment and pharmacologic pain management strategies among registered nurses from a large academic medical center. Subjects were selected using type of nursing unit in which they work and a convenience sampling the study found the majority of participants had bachelor’s degrees (n = 66, 67.3%), 22 (22.4%) had associate’s degrees, and 10 (10.2%) had master’s degrees (5).

Nurses-midwives’ Clinical Practices to Reduce Labor Pains

Table (2) results was consistent with the study for nurses-midwives use in the delivery room the some non-pharmacological methods more than others for different reasons such as decrease nurses-midwives stuff in delivery room, lack of time; no have knowledge and practices about non-pharmacological methods. Inadequate staff served as an obstacle to the utilization the non-pharmacological therapies in managing labor pain. Participants perceived some of the non-pharmacologic approaches to be time consumption for the few attending maternity care providers to administer considering their heavy workload and increased client turnover. Increased workload and client turnover invariably place a lot of stress on the few practicing nurses and midwives, leading to staff exhaustion and impaired work efficiency (2).

Nurses-midwives’ practices to reduce labor pains

Table (3) results show that there are high and moderate mean scores and relative sufficiency in the first, second and third observations in posttest period practices (2, & K); (4); and (8). While other practices not implemented by the nurse-midwives due to the lack in the resources or supplementation in delivery rooms, or due to the routine of the hospitals, or neglect of the nurses-midwives’ to the practice. The results consistent with Boateng et. al, these stated in their study that nurses and midwives were more use some non-pharmacologic approaches such as sacral massage, deep breathing, and frequently use them in their practice to manage labor pain. The frequent usage of these methods has been reported in previous studies and can be attributed to the familiarity of the midwives with these approaches. While insufficient knowledge may account for the decrease frequent use of these methods, other factors such as inadequate human and material resources may have contributed to this observation; similar to what has been reported in other studies (2).
Conclusions
The study concluded that the educational program can be considered as an effective mean for improvement of the nurses-midwives’ practices about the importance pain management.

Conflict of Interest: Nil

Source of Funding: Self

Ethical Clearance: Obtained from the
- Ministry of Health (MOH) - Department of Planning and Health Research Section
- Ministry of Planning - Central Statistical Organization and Information Technology (CSOIT)
- Baghdad Teaching Hospital
- Al-Elwia Maternity Teaching Hospital
- Ibn Al -Balidy for Maternity and Pediatric Hospital
- Fatima Al-Zahra for Maternity and Pediatric Hospital
- Al -Karckh Maternity Hospital
- AL - Yarmouk Teaching Hospital - Maternity Department
- Ethical Permission Obtained from nurses-midwives’

Recommendations: The study recommended to training primary health care nurses in improved pain management is important part of multi-faced approach towards improving and helping women’s to reduce pain during labor.

References
3D CBCT Analysis of Odontometric Variables for Gender Dimorphism in Saudi Arabian Subpopulation

Rakhi Issrani1, Azhar Iqbal2, Mohammad Khursheed Alam3, Namdeo Prabhu4

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Abstract

Background: For assessing the sex of unidentified or skeletonized individuals, the tooth crown diameters are a good and reliable source amongst the other clinical markers.

Aim and Objectives: To check the accuracy and reliability of Mandibular Canine Index (MCI) in sex determination among Saudi subpopulation using 3D Cone Beam Computerized Tomography (CBCT) images. An additional objective is to establish the norm of Standard MCI.

Methodology: A total of 400 CBCT images were studied for the measurements of mesio-distal width (MDW) and inter-canine distance (ICD) of mandibular canines. Then, the observed and Standard MCI were calculated that was statistically determined by receiver operating characteristic (ROC) analysis.

Results: Left canine showed greater sexual dimorphism. The Standard MCI was calculated as 0.239 (right mandibular canine) and 0.241 (left mandibular canine). The ability to determine gender correctly using Standard MCI (right mandibular canine) method was estimated to be 49% among males and 46.5% among females. The accuracy of the method, when applied to the combined data, was 47.8%.

Conclusion: MCI and the variables that allow MCI quantification were shown to distinguish the groups, in terms of average, suggesting the possibility that canine-related measurements may be used for sex discrimination.

Keywords: Canine; CBCT; Odontometric.

Introduction

Forensic odontology plays a vital role in the recognition of victim of mass disasters. Odontometric features used for sex identification are sometimes dependent on the gender wise comparison of tooth dimensions. Among all the teeth, mandibular canines are better-known to show the greatest sexual dimorphism, and so it is assigned as the ‘key teeth’ in human identification.

Odontometric analysis using direct measuring ways has been done using hand-held digital calipers, 2D digital model, graphical illustration to record dimensions on dental casts, digital impression model, 3D CBCT model, and laser scanned model. 3D CBCT has made it possible to conduct odontometric measurements in three-dimension and providing a lot of precise and consistent tools. It conjointly offers additional advantages like availability of images created, decrease in storage expenses and the facility to gauge the pictures by refined software. All these options strengthen the credibility and reliability of 3D CBCT method for the odontometric morphometry.
Despite conducting thorough literature review, no study could be found that has tested this methodology among Saudi population. Hence, the current study was conducted with an aim to examine the accuracy and reliability of MCI in sex determination among Saudi population by in vivo 3D CBCT. An additional objective of this study was to ascertain the norm of Standard MCI.

**Material and Method**

This cross-sectional study of secondary data was conducted between the periods of May 2017 to July 2019. Sample size was assessed using nMaster software (version 2, CMC, Vellore). Anticipating a 60% accuracy of Standard MCI method for predicting gender in the study population, an absolute precision of 5% and a 95% Confidence interval, a sample size of 369 was found to be sufficient. This sample size was rounded off as 400 with equal proportion of males and females.

The data source was CBCT volumetric data from the archives of the College of Dentistry, Jouf University and samples were selected using simple random technique. Odontometric morphometry was analyzed and recorded in 400 3D CBCT volumetric data mandibular arches. The inclusion criteria were i) age-group of 17-35 years; ii) presence and complete eruption of all morphologically normal permanent teeth with exception of third molars; and iii) high quality CBCT that have proper density and contrast with sharp outlines. The exclusion criteria were i) severe crowding; ii) excessive spacing; and iii) interproximal caries or restoration.

**Studied parameters**

**MDW**: measured as the maximum distance between the contact points of mandibular canines on the approximate surfaces of the tooth crown.\(^6\) *(Figure 1)*

**ICD**: measured between the contact points of mandibular canines.\(^7\) *(Figure 2)*

**Procedure**

The selected 400 CBCT images were analyzed using software OnDemand 3D. The readings obtained from measurement MDW and ICD were subjected to mathematical operations to calculate the observed MCI. The formula used to determine canine index was:

\[
\text{Observed MCI} = \frac{\text{MDW of mandibular canines}}{\text{ICD}}.
\]

\[
\text{Standard MCI} = \frac{(\text{mean male MCI} - \text{Standard deviation [SD]}) + (\text{mean female MCI} + \text{SD})}{2}.
\]

The Standard MCI value aided as a cut-off point to differentiate males from females. Individuals with the observed MCI values≤Standard MCI value were categorized as females; otherwise, they were grouped as males.

**Measurement of error**: The readings were repeated at two weeks interval to evaluate the systemic and random errors for 50 CBCT images that were randomly selected. Two-sample *t*-test was used for calculating systemic errors for each pair of analyses. All test and retest measurements presented an intra-class correlation of value greater than 0.91 that is acceptable as per Stirrup (1993).\(^8\) Outcomes form these variables showed that there were no random errors. All pairs of measurements showed a p-value>0.1, suggesting that there was no systemic bias in these analyses as stated by Houston (1983).\(^9\)

**Statistical analysis**

Descriptive analysis of MDW and ICD were presented as means and SD. Predictive accuracy was assessed in terms of percentage. The accuracy of the Standard MCI, to correctly identify the gender, was statistically determined by ROC analysis. Sensitivity and specificity of a diagnostic test can be interpreted by ROC curve that is created by plotting the true positive rate against the false positive rate at various threshold settings. The area under curve (AUC) determines the accuracy of the test. An AUC value of 1.0 indicates the perfect test. If the AUC is 0.9-1.0, it shows excellent test. An AUC value of 0.8-0.9 indicates a good test, whereas an AUC value of 0.7-0.8 depicts a fairly reliable test. The AUC value<0.5 indicate an unreliable test.

**Results**

The descriptive statistics for MDW, ICD and MCI are summarized in Table 1.

1. **MD dimensions**: The mean right and left MD canine dimensions among females were 6.41±0.46mm and 6.48±0.44mm respectively, while among males, the values were significantly higher. This difference was found to be statistically significant (p<0.05).

2. **ICD**: The mean ICD among males was significantly greater than among females.

3. **MCI**: The MCI were found to be higher among
females as compared to males, which was statistically insignificant.

4. Sidewise comparisons: Left canine exhibited greater sexual dimorphism when compared to right canine.

5. Standard MCI: Generally, the Standard MCI is obtained using the right mandibular canine MD dimension. But to check the suitability of left mandibular canine for sex determination, the Standard MCI using the left mandibular canine was also determined. The calculated Standard MCI was calculated as 0.239 (right mandibular canine) and 0.241 (left mandibular canine). The ability to determine sex correctly using Standard MCI (right mandibular canine) method was estimated to be 49% among males and 46.5% among females. The accuracy of the method, when applied to the combined data, was 47.8%. While, when Standard MCI (left mandibular canine) was used as cut-off value, 44.5% males and 48.5% females were correctly predicted. The overall accuracy of this Standard MCI (left mandibular canine) was calculated as 46.5%. (Table 2)

The AUC (for MCI calculated from right mandibular canine) has a value of 0.481 and 0.519 for males and females respectively. The AUC (for MCI calculated from left mandibular canine) has a value of 0.486 and 0.514 for males and females respectively (Figure 3a-d). It can be interpreted that this MCI method was not a reliable method for prediction of gender in the Saudi subpopulation.

Discriminant analysis was utilized to conduct a multivariate analysis of variance test of the hypothesis that males and females would differ significantly on a linear combination of five variables, viz, MDW of right canine, MDW of left canine, ICD, right and left MCI. The overall Chi-square test was significant (Wilks λ=0.696, Chi-square=143.56, df=5, Canonical correlation 0.552, p<0.001). Re-classification of cases based on the new canonical variables was highly successful: 72.3% of the cases were correctly reclassified into their original categories. (Table 3)
Figure 2. Measurement of ICD
Figure 3. ROC curve depicting

a. MCI (as calculated from MDW of right mandibular canine) among males
b. MCI (as calculated from MDW of right mandibular canine) among females
c. MCI (as calculated from MDW of left mandibular canine) among males
d. MCI (as calculated from MDW of left mandibular canine) among females
Table 1: Gender-wise comparison of right & left canine MDW, ICD & MCI values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Gender</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>‘t’ value</th>
<th>‘p’ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lt Canine MDW (mm)</td>
<td>Males</td>
<td>200</td>
<td>6.99</td>
<td>0.49</td>
<td>10.94</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>200</td>
<td>6.48</td>
<td>0.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rt Canine MDW (mm)</td>
<td>Males</td>
<td>200</td>
<td>6.89</td>
<td>0.52</td>
<td>9.88</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>200</td>
<td>6.41</td>
<td>0.46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICD (mm)</td>
<td>Males</td>
<td>200</td>
<td>29.07</td>
<td>2.49</td>
<td>10.01</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>200</td>
<td>26.81</td>
<td>1.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCI (using Rt canine MDW)</td>
<td>Males</td>
<td>200</td>
<td>0.24</td>
<td>0.02</td>
<td>-0.82</td>
<td>0.414</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>200</td>
<td>0.24</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCI (using Lt canine MDW)</td>
<td>Males</td>
<td>200</td>
<td>0.24</td>
<td>0.02</td>
<td>-0.39</td>
<td>0.695</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>200</td>
<td>0.24</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N = Number of participants

*Statistically significant

Table 2: Predictive accuracy

<table>
<thead>
<tr>
<th>MCI</th>
<th>Standard MCI</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%age</td>
<td>N</td>
<td>%age</td>
</tr>
<tr>
<td>Rt mandibular canine</td>
<td>0.239</td>
<td>98</td>
<td>49</td>
<td>93</td>
</tr>
<tr>
<td>Lt mandibular canine</td>
<td>0.241</td>
<td>89</td>
<td>44.5</td>
<td>97</td>
</tr>
</tbody>
</table>
### Table 3: Discriminant function analysis for sex prediction

<table>
<thead>
<tr>
<th>Variables</th>
<th>Raw coefficients</th>
<th>Standardised coefficients</th>
<th>Factor structure matrix</th>
<th>Group centroids</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDW Rt Mandibular canine</td>
<td>14.84</td>
<td>7.29</td>
<td>0.75</td>
<td>0.660 -0.660</td>
</tr>
<tr>
<td>MDW Lt Mandibular canine</td>
<td>-14.52</td>
<td>-6.83</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>MCI - Rt Mandibular canine</td>
<td>-433.42</td>
<td>-8.89</td>
<td>-0.06</td>
<td></td>
</tr>
<tr>
<td>MCI - Lt Mandibular canine</td>
<td>460.17</td>
<td>9.33</td>
<td>-0.03</td>
<td></td>
</tr>
<tr>
<td>ICD</td>
<td>0.54</td>
<td>1.22</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>Constant</td>
<td>-23.79</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

**Classification Results**<sup>a,c</sup>

<table>
<thead>
<tr>
<th>Gender</th>
<th>Predicted Group Membership</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Original**

<table>
<thead>
<tr>
<th>Gender</th>
<th>Count</th>
<th>Male</th>
<th>71.5</th>
<th>28.5</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>143</td>
<td>57</td>
<td></td>
<td></td>
<td>200</td>
</tr>
<tr>
<td>Female</td>
<td>45</td>
<td>155</td>
<td></td>
<td></td>
<td>200</td>
</tr>
</tbody>
</table>

**Cross-validated**

<table>
<thead>
<tr>
<th>Gender</th>
<th>Count</th>
<th>Male</th>
<th>67.5</th>
<th>32.5</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>135</td>
<td>65</td>
<td></td>
<td></td>
<td>200</td>
</tr>
<tr>
<td>Female</td>
<td>46</td>
<td>154</td>
<td></td>
<td></td>
<td>200</td>
</tr>
</tbody>
</table>

---

<sup>a.</sup> 74.5% of original grouped cases correctly classified.

<sup>b.</sup> Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

<sup>c.</sup> 72.3% of cross-validated grouped cases correctly classified.
Discussion

The present study was carried out on CBCT data of 400 patients using the In-Vivo Dental program and the digital models obtained out of 3D onDemand software. The uniqueness of the current study lies upon the following statements: (1) 400 CBCT data; (2) assessment of odontometric morphometry by new approach; and (3) first time in Arabian subpopulation using this novel method.

There is no consensus on the suitability of the MCI in sex determination, as different authors have stated different conclusions. In an Indian sample, Duraiswamy et al. (2009)\(^\text{10}\) claimed 72.0% accuracy rate, while Silva et al. (2016)\(^\text{11}\) mentioned a 54.2% accuracy rate in a Portuguese population. A higher accuracy rate was reported by Yadav et al. (2002)\(^\text{12}\) but a much lower accuracy was stated by Muller et al. (2001)\(^\text{13}\). Variations can, obviously, exist in population disparities.

The present study established that the MD dimension of mandibular canines exhibited statistically significant sex variation. No statistically significant differences were found between right and left mandibular canines in both sexes suggesting that the canine measurement on any side of the jaw can also be used for the other side. This finding is in harmony with the study done by Shahid et al. (2015).\(^\text{14}\) Also a significant difference in ICD was detected between the sexes with male subjects exhibiting larger ICD. Although this finding is consistent with previous reports on the canine index but these measurements were done by digital caliper on plaster model.\(^\text{15}\)

The percentage sexual dimorphisms were also calculated by using Garn and Lewis formula.\(^\text{16}\) The present study found a definite statistically significant sexual dimorphism in mandibular canines, which is consistent with previous studies.\(^\text{17,18}\) The left mandibular canine exhibited greater dimorphism as compared to right. Thus, usage of left mandibular canine for sex determination of an individual will give more precise results. This finding is found to be in accordance with many previous studies showing the values of sexual dimorphism in this range only. Few studies had found a higher degree of sexual dimorphism in canine dimensions while few others had found a lower magnitude of sexual dimorphism. This observation is of definite significance and the discrepancy can be explained based upon the fact that tooth morphology is known to be influenced by environmental, cultural, and racial factors.\(^\text{19}\)

Nonetheless, MCI did not show any statistically significant sexual variation in the present study population. This finding was contradictory to many previous studies.\(^\text{11,18}\) Many recent similar studies on Nepalese and Indian origin population also could not found gender variation in MCI.\(^\text{20,21}\) In addition, discriminant analysis of MCI revealed poor ability to differentiate the sexes. This finding could be attributed to MCI being measured as a relative value- it is estimated as a ratio of two absolute measurements and does not reproduce sex differences that exist in the absolute measurements per se.\(^\text{22}\) Acharya et al. (2011)\(^\text{23}\) in his study have also questioned the reliability of ICD for sex assessment considering the fact that males have larger jaw dimensions with a more predilection for bilobate and square shaped chin in contrast to pointed ones in females. Moreover, MCI relies on ICD, which can be modified by orthodontic treatment or periodontal disease that can result into pathological migration of canines to different positions and despite being very resistant to hazard, teeth particularly a single rooted tooth, can be displaced from its location peri- or post-mortem. In such conditions, methods that rely on having the two mandibular canines in their alveoli cannot be used.\(^\text{24}\)

The ROC curve analyses reported poor discriminative ability values for both right and left MCI, and therefore did not support the use of dental variables for sex estimation.

Conclusion

i. CBCT provides a valuable tool in record and data management for odontometric morphometry assessment obtained directly from the digital image;

ii. MCI and the variables that allow MCI quantification were shown to distinguish the groups, in terms of average, suggesting the possibility that canine-related measurements may be used for sex discrimination; and

iii. The MCI proposed in other population sample was not found to be a reliable tool in sex determination in the present Saudi Arabian sample, and therefore its application should be confined.

Ethical Clearance- Taken from Institutional Ethical Committee (LCBE 9-16/8/39).

Source of Funding- Self-funded.

Conflict of Interest- Nil.
References


Violation of Dapek Salah Customary Law in Bengkulu: Forensic Psychological Analysis Toward The Criminal Behavior of Indigenous

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Abstract

Introduction: Every law is a system which means law bases on the unity of the mind. Customary law is understood as a law that lives in a “living law” society, legal values and a sense of justice that lives in society. All takes place after the values lived by the society under the filter of legal ideals and moral ideals prevailing in the society concerned. Without the law as an effective social control, humans potentially can lead to behavior that can bring destruction to humans. In Bengkulu, the law that lives in the community is known as the law of adat dapek salah. Violation of the law can be acts of a person or group of people who disturb the balance in the indigenous peoples. The violation of the law is an act of deviant behavior that is negative in which each culprit will be charged with various kinds of traditional sanctions. The research of forensic psychology analysis is needed to see the behavior of customary criminal crime in violation of customary law. The assessment is carried out to provide an overview of the mental state of the offender. This is in line with forensic psychology as the study of subjects in terms of cognitive, effective and behavior in relation to the legal process.

Material and Method: This research is a qualitative research which produces descriptive data in the form of written or oral words from people and observed behavior. Qualitative research is also called naturalistic research. This study departs from naturalistic inquiry whose findings are not found from statistical calculations.

Findings: Customary law of Dapek Salah in Bengkulu as a social model directs to accommodate the entire social system which includes the function of norm system. The function of norm system is to correct behavior that deviates from the relevant rules (violations). These rules are the framework of human behavior orientation (members of the social system). Norms are also often referred to as rules of conduct and blueprints for behavior in a society. This can also lead to anti-social behavior or asocial behavior which could be a factor causing criminal behavior. Criminal behavior toward the Dapek Salah law is an act that causes adverse reactions and corrections and the balance of the community. In the study of forensic psychology analysis, criminal behavior on the law depends on the perception of the respective actors. Aspects of perception consist of cognitive aspects (thoughts), affective aspects (feelings), and aspects of conation (behavior). Individual perceptions can influence and motivate subsequent behavior, if the object of perception is judged unpleasant then the behavior is negative.

Conclusion: Violation of the Dapek Salah is a phenomenon, a person or group of people who violate customary law (criminal behavior) so as disturbing the balance in indigenous peoples’ value. In the forensic psychological analysis, criminal behavior of the law depends on the perception of the respective actors. Aspects of perception consist of cognitive aspects (thoughts), affective aspects (feelings), and aspects of conation (behavior). Individual perceptions can influence and motivate subsequent behavior, if the object of perception is judged unpleasant then the behavior is negative. In the basic concept of psychology, there is something wrong with the minds of the perpetrators so that they are involved in criminal behavior. Imbalance relationship between Id, Ego and Superego makes humans weak and consequently more likely to commit deviant behavior or criminal behavior.

Keywords: Violation of Dapek Salah Customary Law, Criminal Behavior, Forensic Psychology
Introduction

Every law is a system which means its rules are unanimity based on the unity of the mind. In Indonesian legal system, there is a customary law. Customary law is understood as a law that lives in a “living law” society, legal values and a sense of justice that lives in society. The unwritten rule of law is determined by the leadership of the alliance, but it comes from habit, code of conduct, from code of conduct to customs, from customs to legal norms. All takes place after the values lived by the community under the filter of legal ideals and moral ideals prevailing in the society concerned.

Law is indeed a human necessity. Without law as an effective social control, human potentially can lead to behavior that can bring destruction to humans, as Lun Fuller said, “human is the only species that chooses its own kind as its preferred prey”. Customary law as a social control system has given its role in the framework of creating community order. Logical consequences as a means of social control, customary law is born, grew and developed in a social system.

Good law is the law in accordance with the law that lives in society. In Bengkulu, the law that lives in the community is known as customary law of Dapek Salah. Violation of the Dapek Salah is a phenomenon, a person or group of people who violate customary law (criminal behavior) so as to disturb the balance in indigenous peoples. At this point, violation of Dapek Salah as a law that lives in the community should not occur. This is because the law is an embodiment of the real legal feelings of the community, it is also a blueprint of behavior that provides guidelines and or benchmarks for community behavior.

In Bengkulu, violation of the customary law can be inevitable wrong called as criminal behavior. Criminality as an act or behavior that is detrimental to the sufferer or victim is also very detrimental to the community in the form of loss of peace and order. Criminality is also an activity which is opposed by the community because it violates the law, social and religion as well as psychological harm. If criminal behavior arises in the form of a violation of customary law, it can be concluded that something has gone wrong with the behavior of the act. Violation of the customary law is an act of deviant behavior in which each culprit will be charged with various kinds of traditional sanctions. A violator is not necessarily identical with chaotic behavior but it could be accidentally or in a desperate condition. Among deviant behaviors, some are triggered by psychiatric conditions that suffer from disorders, mental illness, and others which appear physically healthy. However, there is also based on the growth of the decision to violate the law even though he does not suffer from mental disorders and this is very closely related to the socio-psychological aspects (environment).

Therefore, the study of forensic psychology analysis is needed to see the criminal behavior of customary law in a psychological perspective. The assessment is carried out to provide an overview of the mental of the offender. This is in line with forensic psychology as the study of subjects in terms of cognitive, effective and behavior in relation to the legal process. Based on the above, it is necessary to see how violations of Dapek Salah in Bengkulu City as a study space forensic psychology analysis.

Material and Method

This research is a qualitative research which produces descriptive data in the form of written or oral words from people and observed behavior. Qualitative research is also called naturalistic research. This study departs from naturalistic inquiry whose findings are not found from statistical calculations.

Findings

A. Violations of the Dapek Salah Law in Bengkulu

Dapek Salah customary law as a social model directs to accommodate the entire social system which includes the function of norm system whose task is to correct behavior that deviates from the relevant rules (violations). These rules constitute the orientation framework of human behavior (members of the social system). Norms are one of the basic concepts to explain human behavior and causes of violations. Norms are rules that govern behavior at certain times and situations. Norms are also interpreted as behaviors that are psychologically accepted and expected by the community. Norms are also often referred to as rules of conduct, and blueprints for behavior.
Violation (Dapek Salah Customary Law) has the following principles:\textsuperscript{17}:

1. Violations of customary law or a sense of community justice.
2. The violation will cause a shock in the community’s legal balance, and
3. Customary law sentences to the criminal so that the balance happens in society.

As for the forms of violation of Dapek Salah customary law are taking or damaging other people’s belongings, beating, holding the opposite sex who is not a \textit{muhrim}, committing adultery, traveling with someone who is not a \textit{muhrim}, meeting someone who is not a \textit{muhrim}, doing something unpleasant to a neighbor, disturbing someone’s yard, damaging the environment, embarrass others, and disturb others.

In a social context, criminal behavior is a social behavior that is learned in a community group. This is based on the assumption that humans live in groups and depend on other humans. This terminology can only be applied to humans, and only humans have the capacity to enter into abstract social relations. Without the influence of society, groups of people will not be able to talk and behave socially. Therefore, punishing people in a place for a certain period can produce dramatic acts of behavior. The loss of community and group relations can be a variable that can explain why someone commits criminal behavior. This can also lead to anti-social behavior or asocial behavior which could be a factor causing criminal behavior\textsuperscript{18}.

B. Forensic Psychological Analysis Toward the Criminal Behavior of the Dapek Salah Law in Psychological Perspectives

Criminal behavior in Dapek Salah law is an act that causes adverse reactions, corrections and the imbalance of the community. Criminal behavior is not a hereditary phenomenon that is innate nor inherited biologically. Criminal behavior can be committed by anyone, both men and women, children, adults, and elder. Crimes can occur consciously, that is, think in advance, planned, and directed at a particular purpose. In addition, it can also be done in a semi-conscious, influenced by strong impulses from within a person, strong impulses (compulsions), and obsessions\textsuperscript{19}.

In the study of forensic psychology analysis, the criminal behavior of Dapek Salah law depends on the perception of each actor. Aspects of perception consist of cognitive aspects (thoughts), affective aspects (feelings), and aspects of conation (behavior). Individual perceptions can influence and motivate subsequent behavior. If the object of perception is judged unpleasant, then the behavior is negative\textsuperscript{20}.

In the basic concept of psychology, there is something wrong with the minds of the perpetrators so that they are involved in criminal behavior. The imbalance of the relationship between Id, Ego and Superego makes humans weak and consequently is more likely to commit deviant behavior or criminal behavior. That deviation results from excessive guilt as a result of excessive superego. People with excessive superego will be able to feel guilty without reason and want to be punished. The way people do to deal with guilt is precisely by committing a crime. Crimes are committed to defuse the superego because they unconsciously want a punishment to eliminate guilt. Apart from that, it also can be the principle of “pleasure”. Humans have a biological basis that is urgent and works to achieve satisfaction (pleasure principle). This includes the desire for food, sex, and survival managed by Id. This cannot be obtained legally or in accordance with social rules, so people will instinctively try to do it illegally. Actually, a moral understanding of right and wrong that has been instilled since childhood should be able to work as a superego that counterbalances and controls the Id. But if moral understanding is lacking and the superego does not develop perfectly, a person can grow into an individual who is less able to control the impulse of the Id. This stimulate people to do anything to achieve what their needs. Criminal behavior is not the result of criminal personality, but it comes from ego weakness. An ego that is unable to bridge the needs of the superego and the Id will be weak and make people prone to deviations\textsuperscript{21}.

There are two factors that cause crime (criminal behavior of Dapek Salah law\textsuperscript{22}:
1. Personal factors, including biological factors (age, sex, mental state) and psychological factors (aggressiveness, carelessness, and alienation).
2. Situational factors, including conflict situations and place and time factors.

Criminal behavior is often associated with a bad personality, for example to take revenge against others, often lie to his friends\textsuperscript{23}, narcissistic, aggressive when
their egos are threatened, and intimate relationships with a partner will tend to be problematic because of his selfish attitude and his affair. Psychopathy is also often associated with various forms of crime, including sexual violence and murder.

Naturally, some people struggle to solve social problems through appropriate ways (accepted by the community), such as trying to be individuals who are pleasant and sensitive to the people around them. However, there are also those who choose to use social hostility strategies, which are carried out by individuals with bad characters.

Conclusion

Violation of the Dapek Salah law is a phenomenon, a person or group of people who violate the law so as to disturb the balance in indigenous peoples. If criminal behavior arises in the form of a violation of Dapek Salah law, so it can be concluded that something has gone wrong with the behavior of the act. Violation of the law is an act of deviant behavior that is negative in which each culprit will be charged with various kinds of traditional sanctions. In the study of forensic psychology analysis, the criminal behavior of the law depends on the perception of the respective actors. Aspects of perception consist of cognitive aspects (thoughts), affective aspects (feelings), and aspects of conation (behavior). Individual perceptions can influence and motivate subsequent behavior, if the object of perception is judged unpleasant then the behavior is negative. In the basic concept of psychology, there is something wrong with the minds of the perpetrators so that they are involved in criminal behavior. The imbalance of the relationship between Id, Ego and Superego makes humans weak and consequently is more likely to commit deviant behavior or criminal behavior.

Conflict of Interest: None

Source of Funding: A source of their own funds

Ethical Clearance: None

References

18. Criminology in Perspective of Social Psychology.


The Effect of Marmet Technique on Mother’s Satisfaction in Breastfeeding

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¹Department of Maternity and Pediatrics, Faculty of Nursing, Universitas Airlangga

Abstract

Background: The Marmet technique is a safe way to stimulate the breast to produce more milk. The administration of Marmet techniques to mothers who produced little to no amount of breast milk showed good results. Palms and fingers are recommended for breast milk milking due to them being practical, effective and efficient.

Purpose: To determine the effect of Marmet technique on maternal satisfaction in breastfeeding.

Method: This research applied quasi experiment design. There were 40 respondents, each consisting of a treatment group and a control group that met the inclusion criteria. Data were collected using questionnaires and observation sheet. The data were then analyzed using Mann Whitney and Wilcoxon’s Test (α <0.05).

Results: Statistical test results of Wilcoxon Signed Rank Test in the treatment group with the value of sig (2-tailed) obtained p = 0.000 in which p <0.05 indicates that there is a significant effect of Marmet technique on maternal satisfaction in breastfeeding. However, the control group obtained p = 0.083 in which p > α > 0.05, meaning that there is no significant effect of Marmet technique on maternal satisfaction in breastfeeding.

Conclusion: The satisfaction of the mother in breastfeeding has increased after being intervened with Marmet technique.

Keywords: Marmet technique, maternal satisfaction, breastfeeding.

Introduction

Breastfeeding is seen to be one of the solutions that may reduce infant mortality, risk of stunting, obesity, and chronic diseases. Babies who were breastfed have a fourteen times bigger chance of surviving in their first six months compared to those who are not breastfed. Failure in breastfeeding is mostly caused by the infants having difficulties in sucking, abnormalities of the breast, and the mother’s lack of knowledge about the breastfeeding. Those failures tend to cause the mothers to stop the process of breastfeeding, and start giving formula milk as a substitute for breast milk. This is due to the mothers’ limited knowledge on breast massaging, which merely revolves around the usual method, oxytocin massage.

One of the factors that cause in infants is the cessation of breast milk in less than 6 months, which is considered to early, and then continued with formula feeding. The percentage of babies who are breastfed and do not experience diarrhea is higher than those who are not. The production of breast milk can be increased by using several methods including oxytocin massage, Marmet technique, warm compresses, back massage, breast care, endorphins and oxytocin massage stimulation and suggestive provision, but due to the limited information in health services regarding the procedure of implementation-these methods are merely known and are rarely given by nurses. One way to extract milk is through Marmet technique, a combination of milking and massaging the breasts in postpartum mother. The administration of Marmet techniques to mothers who
produced little to no amount of breast milk showed good results. The Marmet technique is a safe way to stimulate the breast to produce more milk. If Marmet technique is done effectively, the milk production will be better and thus cause more satisfaction in breastfeeding8,9.

Self-satisfaction of the mother is a cognitive aspect of subjective well-being. Metnal self-satisfaction can be seen from two different approaches, first the bottom-up theory in which self-satisfaction is influenced by an individual’s assessment of important domains in herself. The second is the top-down theory in which the self-satisfaction of the mother will affect another person’s satisfaction domain. Someone who is satisfied with herself will also evaluate important domains in life more positively, although self-satisfaction in general is not only based on satisfaction with that domain alone. Mothers who are able to breastfeed their babies tend to be satisfied6.

**Method**

This study is a quasi-experimental research. The population in this study were breastfeeding mothers in the working area of the Ampenan Puskesmas (Community Health Center) located in six different integrated healthcare centers. The sample selection used inclusion criteria as follows: (1) Mothers who do not exclusively breastfeed (2) Have 3-6 months old babies (3) Able to read and write. The number of samples were divided into the control group and the treatment group. Sampling was done using probability sampling in the method of cluster sampling. The independent variable in this study is the Marmet technique, while the dependent variable is maternal satisfaction in breastfeeding. Prior to the research, both groups were pre-tested. The treatment group was treated with Marmet technique (every day for 20-30 minutes) and the control group was given a counseling about breast massage (for 30 minutes) then both were assigned for a post-test and the data were analyzed using Wilcoxon and Mann-Whitney test.

Satisfaction measurements was done based on a modification of the Breastfeeding Self Efficacy Scale - Short Form (BSES-SF) scale. BSES-SF is a questionnaire that contains 14 items statements about mothers’ confidence in breastfeeding. Validity test was carried out using Pearson product moment (r). $r$ value was calculated with the value of $r$ table at a significance level of 5% and was declared valid. The reliability test in this research was based on Alpha Cronbach, in which the basis for decision making is reliable if the value of alpha $> r$ table. Based on the questionnaire reliability test, the value of Alpha Cronbach for the satisfaction questionnaire was 0.687. This indicates that the questionnaire is reliable. This research has been declared “Ethically Feasible” with a Certificate of Ethical Feasibility from the Faculty of Nursing, Universitas Airlangga, Surabaya, Indonesia.

**Results**

**Characteristics of Respondents**

The table below explains the respondents characteristic.

**Table 1. Characteristics of Respondents**

<table>
<thead>
<tr>
<th>General Data</th>
<th>Category</th>
<th>F</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>21-24</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>25-28</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>29-31</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>32-35</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Junior High School</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>High School</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>University</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>40</td>
<td>40</td>
</tr>
</tbody>
</table>
## Maternal Satisfaction of Breastfeeding Mothers in the Treatment Group Prior to the Introduction of Marmet Technique

Breastfeeding mothers in the treatment group were proven to be dissatisfied in all components of satisfaction in the questionnaire prior to the introduction of Marmet technique.
<table>
<thead>
<tr>
<th>Satisfaction Component</th>
<th>Number of Respondent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dissatisfied</td>
</tr>
<tr>
<td>Duration of breastfeeding</td>
<td>72.5</td>
</tr>
<tr>
<td>Breastfeeding adjustment</td>
<td>72.5</td>
</tr>
<tr>
<td>Mother’s dissatisfaction</td>
<td>82.5</td>
</tr>
<tr>
<td>Breastfeeding satisfaction</td>
<td>80.0</td>
</tr>
<tr>
<td>Time management dissatisfaction</td>
<td>70.0</td>
</tr>
<tr>
<td>Breastfeeding experience dissatisfaction</td>
<td>67.5</td>
</tr>
<tr>
<td>Breastfeeding with family satisfaction</td>
<td>77.5</td>
</tr>
<tr>
<td>Satisfaction due to the will to breastfeed the child</td>
<td>80.0</td>
</tr>
<tr>
<td>Satisfaction in breastfeeding the baby while he/she is</td>
<td>85.5</td>
</tr>
<tr>
<td>crying</td>
<td></td>
</tr>
<tr>
<td>Satisfaction due to breastfeeding in general</td>
<td>87.5</td>
</tr>
<tr>
<td>Satisfaction due to the baby sucking properly</td>
<td>87.5</td>
</tr>
<tr>
<td>Satisfaction due to exclusive breastfeeding</td>
<td>95.0</td>
</tr>
<tr>
<td>Satisfaction after being able to breastfeed</td>
<td>95.0</td>
</tr>
<tr>
<td>Satisfaction due to the baby receiving enough milk</td>
<td>100.0</td>
</tr>
</tbody>
</table>
### Table 3: Maternal Satisfaction of Breastfeeding Mothers in the Control Group Prior to the Introduction of Marmet Technique

<table>
<thead>
<tr>
<th>Satisfaction Component</th>
<th>Number of Respondent (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dissatisfied</td>
<td>Satisfied</td>
</tr>
<tr>
<td>Duration of breastfeeding</td>
<td>100.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Breastfeeding adjustment</td>
<td>100.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Mother’s Dissatisfaction</td>
<td>95.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Breastfeeding satisfaction</td>
<td>90.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Time management dissatisfaction</td>
<td>75.0</td>
<td>25.0</td>
</tr>
<tr>
<td>Breastfeeding experience dissatisfaction</td>
<td>70.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Breastfeeding with family satisfaction</td>
<td>70.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Satisfaction due to the will to breastfeed the child</td>
<td>57.5</td>
<td>42.5</td>
</tr>
<tr>
<td>Satisfaction in breastfeeding the baby while he/she is crying</td>
<td>62.5</td>
<td>37.5</td>
</tr>
<tr>
<td>Satisfaction due to breastfeeding in general</td>
<td>82.5</td>
<td>17.5</td>
</tr>
<tr>
<td>Satisfaction due to the baby sucking properly</td>
<td>92.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Satisfaction due to exclusive breastfeeding</td>
<td>85.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Satisfaction after being able to breastfeed</td>
<td>77.5</td>
<td>22.5</td>
</tr>
<tr>
<td>Satisfaction due to the baby receiving enough milk</td>
<td>80.0</td>
<td>20.0</td>
</tr>
</tbody>
</table>

**Maternal Satisfaction of Breastfeeding Mothers in the Treatment Group Prior to the Introduction of Marmet Technique**

After the application of Marmet technique, breastfeeding mothers in the treatment group were 100% satisfied.
### Table 4: Maternal Satisfaction of Breastfeeding Mothers

<table>
<thead>
<tr>
<th>Satisfaction Component</th>
<th>Number of Respondent (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dissatisfied</td>
<td>Satisfied</td>
</tr>
<tr>
<td>Duration of breastfeeding</td>
<td>0.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Breastfeeding adjustment</td>
<td>0.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Mother’s Dissatisfaction</td>
<td>0.0</td>
<td>97.5</td>
</tr>
<tr>
<td>Breastfeeding satisfaction</td>
<td>0.0</td>
<td>97.5</td>
</tr>
<tr>
<td>Time management dissatisfaction</td>
<td>0.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Breastfeeding experience dissatisfaction</td>
<td>0.0</td>
<td>97.5</td>
</tr>
<tr>
<td>Breastfeeding with family satisfaction</td>
<td>0.0</td>
<td>97.5</td>
</tr>
<tr>
<td>Satisfaction due to the will to breastfeed the child</td>
<td>0.0</td>
<td>97.5</td>
</tr>
<tr>
<td>Satisfaction in breastfeeding the baby while he/she is crying</td>
<td>0.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Satisfaction due to breastfeeding in general</td>
<td>0.0</td>
<td>90.0</td>
</tr>
<tr>
<td>Satisfaction due to the baby sucking properly</td>
<td>0.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Satisfaction due to exclusive breastfeeding</td>
<td>0.0</td>
<td>95.0</td>
</tr>
<tr>
<td>Satisfaction after being able to breastfeed</td>
<td>0.0</td>
<td>92.5</td>
</tr>
<tr>
<td>Satisfaction due to the baby receiving enough milk</td>
<td>0.0</td>
<td>87.5</td>
</tr>
</tbody>
</table>
Table 5: Maternal Satisfaction of Breastfeeding Mothers in the Control Group After the Introduction of Marmet Technique

<table>
<thead>
<tr>
<th>Satisfaction Component</th>
<th>Number of Respondent (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dissatisfied</td>
<td>Satisfied</td>
</tr>
<tr>
<td>Duration of breastfeeding</td>
<td>100.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Breastfeeding adjustment</td>
<td>100.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Mother’s Dissatisfaction</td>
<td>95.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Breastfeeding satisfaction</td>
<td>90.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Time management dissatisfaction</td>
<td>75.0</td>
<td>25.0</td>
</tr>
<tr>
<td>Breastfeeding experience dissatisfaction</td>
<td>70.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Breastfeeding with family satisfaction</td>
<td>70.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Satisfaction due to the will to breastfeed the child</td>
<td>57.5</td>
<td>42.5</td>
</tr>
<tr>
<td>Satisfaction in breastfeeding the baby while he/she is crying</td>
<td>62.5</td>
<td>37.5</td>
</tr>
<tr>
<td>Satisfaction due to breastfeeding in general</td>
<td>82.5</td>
<td>17.5</td>
</tr>
<tr>
<td>Satisfaction due to the baby sucking properly</td>
<td>92.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Satisfaction due to exclusive breastfeeding</td>
<td>85.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Satisfaction after being able to breastfeed</td>
<td>77.5</td>
<td>22.5</td>
</tr>
<tr>
<td>Satisfaction due to the baby receiving enough milk</td>
<td>72.5</td>
<td>27.5</td>
</tr>
</tbody>
</table>

Differences in the Satisfaction of Breastfeeding Mothers in the Treatment and Control Groups Before and After the Application of Marmet Technique
Table 6: Statistical Test Results

<table>
<thead>
<tr>
<th>Category</th>
<th>Treatment</th>
<th></th>
<th></th>
<th>Control</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pretest</td>
<td>Post test</td>
<td>Pretest</td>
<td>Posttest</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>f</td>
<td>%</td>
<td>F</td>
<td>%</td>
<td>f</td>
<td>%</td>
</tr>
<tr>
<td>Dissatisfied</td>
<td>40</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>40</td>
<td>100</td>
</tr>
<tr>
<td>Satisfied</td>
<td>0</td>
<td>0</td>
<td>40</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Very Satisfied</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Σ</td>
<td>40</td>
<td>100</td>
<td>40</td>
<td>100</td>
<td>40</td>
<td>100</td>
</tr>
</tbody>
</table>

Wilcoxon Signed Rank Test

<table>
<thead>
<tr>
<th></th>
<th>p : 0.000</th>
<th>α≤0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mann Whitney U Test</td>
<td>p : 0.000</td>
<td>α≤0.05</td>
</tr>
</tbody>
</table>

Discussion

Based on the results of the study, it can be from the pretest that prior to the intervention in the form of a Marmet technique, the respondents in the treatment group expressed that they were dissatisfied in every category. The majority of the respondents, or 18 respondents (45%), are either self-employed or entrepreneurs. Working mothers tend to be less focused on caring for babies and their families, causing satisfaction in breastfeeding to be reduced as well compared to mothers who are not working. Most mothers, in breastfeeding their first child, were more focused with their work in order to help their husbands in meeting their daily needs. Thus, their children were entrusted to distant relatives or neighbors and provided with formula milk\(^{10,11}\).

The pretest that prior to the intervention of Marmet technique, the respondents in the control group expressed that they were dissatisfied in every category. Most respondents were mothers with second children as much as 18 respondents (45%). Mothers with two or more children already have an experience in breastfeeding and caring for their babies compared to mothers who just gave birth the their first children or only have with one child. This is due to mothers not yet developing experience in taking care of their first children, especially in breastfeeding\(^{12}\). As seen from the posttest that after the intervention in the form of a Marmet technique, the respondents in the treatment group expressed that they were satisfied in every category. The majority of respondents in this study were in the 21-35 year age group, also known as the productive age. Age is one of the physiological factors that can directly influence the process of breastfeeding\(^{13}\).

The posttest that after the intervention of Marmet technique, the respondents in the control group expressed that they were dissatisfied in every category. The results of the study show that the maternal satisfaction in breastfeeding in the control group was influenced by the respondents’ education levels. A total of 22 respondents (55%) graduated from elementary school or junior high school. Education is one of the factors that indirectly affects the satisfaction of mothers in breastfeeding since it is related to their socio-cultural background. This factor has a correlation towards the mother’s ability to receive information that will later affect the process of breastfeeding. A mother who has an adequate knowledge will have an increased satisfaction in breastfeeding.

Statistical test results of Wilcoxon Signed Rank Test showed a significant increase in the satisfaction scores in breastfeeding. The total of respondents who experienced an increase in the satisfaction scores in the treatment group were 40 respondents. There are 9 respondents whose satisfaction in breastfeeding increased to the category
of very satisfied. While respondents whose satisfaction increased to the satisfied category were 31 respondents. The satisfaction of the mother in breastfeeding with the intervention of the Marmet technique is influenced by the technique of massaging and milking the breasts. The recommended milking technique is to use hands, palms, and fingers because it is practical, effective and efficient compared to using a pump. The process include *massaging, stroking*, and *shaking* the breast in order to stimulate the milk reflex.\(^{15}\)

The satisfaction of the mothers after the application of the Marmet technique was seen to increase drastically, especially in respondents in the treatment group. Respondents who applied the Marmet technique felt more satisfied in breastfeeding their baby. Respondents felt the impact of the intervention gradually and experienced an increase in satisfaction, such as satisfaction of the mother for successfully breastfeeding her baby, and satisfaction because the baby had received enough milk. The referred respondents were breastfeeding mothers in the treatment group who applied Marmet technique routinely and were monitored by researchers. The researcher also explained to the respondent that if there were complaints of pain in the application of Marmet technique, it was advisable to temporarily stop the technique and take immediate action such as compressing the painful area. This is done to immediately solve the problem that may arise and the evaluation process can continues to run according to a predetermined schedule, the flow of milk that increased production may meet the needs of the baby, and the use of formula milk can be reduced as early as possible.

**Conclusion**

Satisfaction in the treatment group after the intervention was seen to increase compared to the control group. Marmet technique can increase the satisfaction of mothers in breastfeeding due to the stimulation of the breast muscles from the massage and milking that is done to trigger the milk reflex, causing the mother to feel satisfied because the production of breast milk is increasing.

**Ethical Clearance:** Taken from Faculty of Nursing Universitas Airlangga, Surabaya ethic committee

**Source of Funding:** This research was founded by author.

**Conflict of Interest:** None

**Reference**

1. Lawrence RA, Lawrence R. Breastfeeding: A guide for the medical profession, Mosby, St. Louis MO. 2005;


The Increase Level of Muscle Adductor in Idiopathic Vocal Cord Adductor Paralysis Post Biofeedback Vocal Therapy

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The Department of Otolaryngology, Faculty of Medicine, Universitas Airlangga, General Hospital of Dr. Soetomo Teaching Hospital, Surabaya, 60285, Indonesia

Abstract

**Background:** The Idiopathic Vocal Cord Adductor Paralysis (KAKVI) is the inability of muscle that moves the vocalist notochord to medial in phonation time with the idiopathic. This condition might has impact to the emerge of hoarse voice and other lamentations which are related to the voice production. The conventional voice therapy still show the number of the recovery of low dysphonia.

**Method:** The diagnosis of KAKVI is based on the anamnesis, physical check up, thorax photograph and laryngoscopy fiberoptic and is conducted the measurement of vocalist cord adductor muscular construction strength before the BF voice therapy with equipments. Then, the BF voice therapy is conducted twice a week for four weeks. The voice exercise is continued in home once a day by patients themselves. The measurement of the strength of vocalist cord adductor muscle contraction is conducted after the BF voice therapy.

**Results:** The result of the study revealed that there were KAKVI. The result of adductor muscular contraction strength with statistic examination in a short test is \( p = 0.044 \). The result of statistic examination of long is \( p=0.000 \). The result of statistic test of count test is \( p=0.000 \). Therefore, the change of adductor muscular contraction strength on KAKVI after the BF voice therapy is found a significant increase (\( p < 0.05 \)).

**Conclusion:** This study revealed that there are an increase of adductor muscular contraction strength in KAKVI after the BF voice therapy.

**Keywords:** The strength of adductor muscle contraction, idiopathic vocalist cord adductor paralysis, voice therapy, Biofeedback.

Introduction

The paralysis of idiopathic vocalist cord adductor (KAKVI) is a muscular inability to move the vocal cord to medial on phonation time with an idiopathic cause which is the cause is undetected or the pat physiology. This condition can lead the emerge of hoarse voice and other lamentations related with the voice production \(^1\)–\(^5\).

The KAKVI therapy consists of the nonsurgical therapy and surgical therapy. The nonsurgical therapy is the conventional voice therapy, then if it fails or there is particular indication, it can be taken into consideration with the surgical therapy \(^1\)–\(^3\). At the Otolaryngology Outpatient Unit in Dr. Soetomo hospital, the conventional voice therapy on dysphonic still show low recovery number. This low recovery number might be caused by the low self-motivation of the patients to practice and control routinely \(^4\)–\(^5\). The dysphonia research used other therapy methods which is expected to be more effective is the Biofeedback (BF) voice therapy. Biofeedback is the equipment to assist the patients to control voluntarily the strength of muscular contraction that is joined the vocal cord adduction process. However, the increase of adductor muscular contraction strength in KAKVI after the BF voice therapy is still unclear \(^6\)–\(^10\).

The KAKVI patients at the Otolaryngology Outpatient Unit of Dr. Soetomo hospital is 176 (62.4%) of 282 patients (year 1995-1999) and 73 (54.07%) form 135 patients (year 2006-2010). The KAKVI therapy at Otolaryngology Out-patient Unit of Dr. Soetomo
hospital is the conventional voice therapy, with the low recovery number of 19.6\% and 25.56\% \cite{4,5}.

The BF voice therapy requires the electronic equipments which is the *laryngeal electromyography* (LEMG) through the electrode that is put in the surface, so it is called *surface laryngeal electromyography* (sLEMG) that shows the strength of larynx muscular contraction. The patients are asked to see the computer monitor to receive information about what happened in the their skins. The patients are asked to do the various kinds of practices under the voice therapist guidance while seeing the monitor (the feedback is visual signal) or listen to the beep voice (the feedback is auditory signal) \cite{7,11,12}.

The BF voice therapy is started to be reported in the literature with a good result, that is the improvement of vocal cord deviation. According to the previous study, it was reported that 21 normal persons and 7 patients with vocal nodule who underwent the BF therapy session shows that there are a significant difference in EMG score, and the decrease level of muscle tension \cite{13}. It revealed that there were positive correlation between the EMG result with the voice quality assessment.

Another study reported that the use of sLEMG thyroarytenoid muscle in 25 patient, is an efficient and objective test to provide the prediction of the recovery after facing a vocal cord paralysis. In this study, wants to prove the increase point of adductor muscle contraction on KAKVI after the BF voice therapy.

### Material and Method

This study was conducted on June until August 2015 at the Otolaryngology Out-patient Unit and Medical Rehabilitation Instalation of Dr. Soetomo hospital, Surabaya. The study is about the change of the adductor muscle contraction strength in the idiopathic vocal cord adductor paralysis (KAKVI) after the BF voice therapy. The inclusion criteria of subject of study is KAKVI patients who diagnosed under the fiber optic laryngoscopy, attained the age of 20-60, understand the guidance and able to answer questions well, have the ability to control voluntarily, have self-movitation to coordinate well, willing to join the study, and sign the agreement letter in joining the study.

During the study period, it was obtained nine KAKVI patients who got the BF voice therapy twice a week in four weeks. The consecutive sampling is applied in this study \cite{14}. The type of the study is pre experimental with the pre post design without control group. The measurement of adductor muscle contraction strength is conducted pre and post the BF voice therapy. In the BF voice therapy, the patients is asked to breathe in first, then say a long a in one breath, then do a count test in 1,2, 3, etc. in one breath with saying a loudly (short a) as long as the patients is capable.

### Results

#### Table 1. Age Distribution

<table>
<thead>
<tr>
<th>Age (year)</th>
<th>Quantity</th>
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</tr>
</thead>
<tbody>
<tr>
<td>31-40</td>
<td>2</td>
<td>22.22</td>
</tr>
<tr>
<td>41-50</td>
<td>5</td>
<td>55.56</td>
</tr>
<tr>
<td>51-60</td>
<td>2</td>
<td>22.22</td>
</tr>
<tr>
<td>Total</td>
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<td>100</td>
</tr>
</tbody>
</table>

#### Table 2. Gender Distribution

<table>
<thead>
<tr>
<th>Gender</th>
<th>Quantity</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>2</td>
<td>22.22</td>
</tr>
<tr>
<td>Female</td>
<td>7</td>
<td>77.78</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>100</td>
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</tbody>
</table>
Table 3. Job Distribution

<table>
<thead>
<tr>
<th>Job</th>
<th>Quantity</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seller</td>
<td>4</td>
<td>44,45</td>
</tr>
<tr>
<td>Sailor</td>
<td>1</td>
<td>11,11</td>
</tr>
<tr>
<td>Operator</td>
<td>2</td>
<td>22,22</td>
</tr>
<tr>
<td>Teacher</td>
<td>2</td>
<td>22,22</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>100</td>
</tr>
</tbody>
</table>

The core data of the study included the ages, gender, jobs, and the clinic characteristic of the KAKVI patients who obtained the BF voice therapy. The most age group that was 41-50 years was five patients (55,56%). The youngest age was 31 years old, and the oldest one is 59 years old (table 1). The most gender is female which was seven patients (77,78%). The comparison between male and female is 1:3,5 (table 2). The most jobs is seller that are four patients (44,45%) (table 3). The vocal cord that were mostly encountered paresis is the right vocal cord that were five patients (55,55%) (table 4).

The measurement of the adductor muscle contraction strength in KAKVI pre and post the BF voice therapy. The strength of adductor muscle is measured by electromiograph which was completed by BF. The adductor muscle contraction strength is measured when the patients did the short a test, while the adductor muscle contraction endurance is measured when the patients did the long a test and count test. The strength of adductor muscle contraction when the patients did the short a test can be seen in table 5. The minimal average of the adductor muscle contraction strength is when the patients did the short a test before the BF voice therapy is 23,44 µvolt and post therapy is 17,66 µvolt. The maximal average of the contraction strength pre therapy is 43,44 µvolt and post therapy is 55,33 µvolt. The delta average or the gap between the maximal contraction strength and minimal contraction strength is 21,11 µvolt and post therapy is 37,66 µvolt. The statistic examination with the t pairing sample test is obtained p= 0,044. It means that there is a meaningful difference between the minimal contraction strength and the maximal adductor muscle pre and post BF voice therapy (p<0,05).

The endurance of adductor muscle contraction can be seen in the long a test and count test. The endurance of adductor muscle contraction in a long a test is shown in table 6. The minimum average endurance of the adductor muscle contraction when the long a test is conducted based on seconds pre BF voice therapy is 6,22 seconds and post therapy is 18,55 seconds. The statistic examination with the t pairing sample is obtained p=0,000. It means that there is a meaningful difference between the minimal contraction endurance period and the maximal adductor muscle in the long a pre and post the BF voice therapy. The minimum average of the adductor muscle contraction endurance when the count test is conducted based on seconds the pre BF voice therapy is 7,33 seconds and post therapy is 23,77 seconds. The statistic examination with the t pairing sample examination is obtained p=0,000. It means that there is a meaningful difference between the minimal contraction endurance period and maximal adductor muscle in pre and post BF voice therapy count test (p<0,05).

Discussion

Many literatures stated that the vocal cord paralysis often encounter in the left side because the left vocal cord journey is much longer than the right side so it is easier to be affected by lesions. This condition is often caused by idiopathic, not because of inflammation, tumor, trauma, or neurological nuisance. This study revealed the number of KAKVI varied, 60% of the patients encountered the left vocal cord adductor paralysis and 40% of the patients encountered the right vocal cord adductor paralysis which cannot be known the causes.

A meaningful difference or change is better between the pre and post BF therapy; it is because the high level of the patients' self-motivation in producing a sound when seeing the practice result in the monitor. The repeated practice or muscle re-education aims to obtain the
feedback that will control the neuromuscular; increase the muscle ability or muscle ability to tense, restore the agonist action/antagonist of normal muscle, and train the postural control. The patients used to to produce a sound with the better muscle strength 10,15,16.

In six patients with excessive laryngeal tension is conducted with giving 14 BF sessions in 30 minutes each session. At the end of every therapy, three of six patients encountered the reduction of laryngeal EMG level and their voice quality are getting better. Those mentioned improvement happened because the patients are suggested to see the contraction muscle in the monitor then the therapists facilitated the contraction with electric signal in the intended muscle so that the patients can practice well. The other two patients only encountered the change in the EMG level and there is no change in the voice quality. In these two patients, it is actually revealed that there are the heavy structure damage, so it is suggested that the BF therapy might be useful in the functional dysphonia patients only 17.

To be normally voiced, not only the anatomy and cardiopulmonary are needed, but also the good physiological factor. In the KAKVI patients in this study, it is revealed that the adductor muscle that is unilateral, so that one of vocal cords cannot move to median and docked in a median line. The vocal cord paralysis is also affecting the vocal cord tension is lowered, so the vocal cord vibratio will also be disturbed 18. The long a test is actually similar with the MPT test (maximum phonation time) that is often known in THT KL. Maximum Phonation Time is mostly related with the pulmonary condition while in this study, there is no deviation in lungs (idiopathic), so the long a test is mostly used to see the maximum vocal cord contraction 16.

The other supported researches stated that 21 normal persons and seven patients with vocal nodule that undergo eight practice sessions with BF showed that there are a significant difference in LEMG score, and the reduction of muscle tension level. The reduction of muscle level happened because the chosen biofeedback for relaxation practice is high sensitivity setting, therefore that the small electricity activity is much easier to detect. When the relaxation is started to increase, the sensitivity setting is increased from low to high. This change caused the patients must relax more vocal cord muscles so that the relaxation is easier to reach. Moreover, there is a positive correlation between the LEMG result and the vocal quality assessment 19.

In the long a test, the patients were asked to say a after breathing, until the they were out of breath, while there is a respite in count test when the patients said numbers until they were out of breath. The other researches that support showed that the use of LEMG in musculus tiroaritenoid in 25 vocal cord paralysis patients, use the efficient and objective equipment can predict about the recovery after suffering from vocal cord paralysis. This is said because the biofeedback practice can increase the comprehension of how the effort in producing sound and patients can handle the vocal cord muscle relaxation response. In the initial session with biofeedback, the patients can centralize their attention to the feedback and try to handle the sound or visual. When the patients paid attention to the relaxation, they will be able and succeed to handle the sound better 20. Other suitable researches conducted the BF combination with other types of BF with targeting not only in tension and larynx muscle but also the relaxation in general. The use of BF in other voice deviation is rarely obtained, such as ventricular fold dysphonia and paradoxical vocal fold motion, is reported to give the good result 5.

The result of BF measurement can be observed, amplified, and transformed, so that it can give feedback about the condition of patients body in a form of visual signal, particularly in graphic and beep voice that can be understood. The signal is applied to be the guidance to manipulate the signal that appeared in the BF equipment. The patients can learn to control to handle their condition, and make changes, such as relaxation or strengthen the particular tonus muscle. This technique requires the patient’ willingness to conduct the signal change in order to reach their aims. The BF therapy is defined as particular therapy technique that gives the patients strengths to use their mind to control their body and practice to improve their health by using the signal form their own bodies. The mentioned improvement can be reached if the patients see the contraction muscle in a monitor, then the therapist facilitated contraction with electrical signal in the intended muscle, so that the patients can practice to tense the muscle well 7,9-12,21.

Conclusion

It can be concluded that there is an increase in adductor muscle contraction strength in the idiopathic vocal cord adductor paralysis (KAKVI) post biofeedback voice therapy.
Conflict of Interest: There is no conflict interest

Source of Funding: This study is self-funded

Ethical of Clearance: This study was approved by Ethical Commision of Health Research Faculty of Medicine University of Airlangga

Reference

Periodontal Evaluation of One-Piece and Two-Piece Single Implant Loaded in Esthetic Zone

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Abstract

Background: Soft tissue around dental implant is an important anatomical feature contributing to the long-term implant success and esthetics, different factors may influence the soft tissue-implant interface. The purposes of the current study were to evaluate the soft tissue profile around one-piece and two-piece implants loaded in esthetic zone.

Method: Totally thirty patients who had single missing tooth in premaxillary region were included in the study. Fifteen patients’ group-A were assigned to immediate loading one-piece implant and fifteen patients’ group-B were assigned to early loading two-piece implant protocol. Flapless approach was performed for both groups. In group-A, implants were loaded immediately with a temporary crown within 48h, while group-B, were loaded after two months following conventional impression for metal ceramic crown. Clinical outcomes were evaluated after two and six months in terms of success rate, papillary index, plaque index and width of keratinized mucosa.

Results: The success rate in group-A found to be 80%, which was lower than the success rate in group-B (100%). On comparison, there was no statistically significant difference in success rate between the two study groups. There was no statistically significant difference between both groups over time in clinical parameters like plaque index, width of keratinized gingiva and papillary index.

Conclusion: The results of this study indicated that using either immediately loading one-piece implants or early loaded two-piece implants protocol demonstrated the same enhancements of implant esthetics.

Keywords: One-piece implant, Two-piece implant, immediate load, Esthetic zone.

Introduction

Rehabilitation of missing teeth in the anterior maxilla with an implant-supported fixed prosthesis is a broadly accepted treatment modality¹. Many research studies have been done in implantology correlated to implant design, surgical technique, immediate implant placement and loading protocols. Traditionally, implants were subjected to delay load healing for several months, letting the implant to osseointegrate without being exposed to external forces.

Over the last few years, the concept of immediate loading or early loading flapless implant protocol has gained attention. This concept is defined as the application of a load by means of an occluding or a non-occluding restoration within 48 h for immediate loading and 48h to two months for early loading implant placement ².

Patients with a lost anterior tooth may benefit from immediate loading. Placement of the temporary crown after implant placement reduces the total treatment time, avoids a second-stage operation and offers immediate comfort during healing stage. Immediate loading, has some disadvantages, for example, might induce micromotion and instability of the implant.³,⁴

The surgical protocol for implant placement consists of both flap and flapless procedures. Reduced postoperative bleeding, fewer discomfort for the patient, shorter surgery time, and decreased healing time are reported advantages for the flapless procedure compared to flap surgery ⁵,⁶.
Lately, a new description of implant success criteria was proposed, according to which implant success should be detected by using composite outcome measures, including patient-reported outcome measures, peri-implant tissue health and functional and esthetic outcomes related to implant-supported reconstruction case-control and cohort.

An early effort to assess the esthetic aspects of dental implants was made in 1997 by Torsten Jemt who proposed a papilla index, which evaluated the size of the interproximal papilla.

Meanwhile, successively described assessment methods of esthetic results have been subjected to several studies aiming to test the accuracy and effectiveness of these methods. Nowadays, numerous factors in addition to the size of interproximal papilla including the color, form, and the level of peri-implant soft tissues have been included in evaluation of the esthetic outcomes.

The purpose of this study was to evaluate the soft tissue profile of one-piece and two-piece single loaded implant in esthetic zone.

**Materials and Methods**

**Patients and Methods**

For this randomized clinical trial, 30 patients requiring single-tooth implant in the premxillary region are going to be recruited at the periodontology department-College of Dentistry- Hawler Medical University in Erbil city. The subjects randomly assigned to one of two groups: One-piece implant -OPI- group-A (15 patients) or Two pieces implant -TPI- group-B (15 patients). Group-A had their implants loaded immediately within 48h with temporary crown which are out of occlusion, and temporary crown replaced with permanent crown two months later, while group B had implants with early loading two months after implant placement, then follow-up period started: the first assessment done at the day of permanent crown insertion and the second assessment performed six months after loading permanent crown. Informed consent obtained from all subjects, and the use of human subjects in this project was under the approval of the Ethics committee in College of Dentistry. The study period (from patient enrollment to data collection) was between November 2018 and July 2019.

**Patient selection**

Subjects who lost single tooth in premxilla at least three months of post-extraction healing were selected to participate in the study. Entry criteria includes: periodontal healthy subjects; age 18-60 years old; agreed to follow up visits for 6 months; controlled oral hygiene; absence of any lesions in the oral cavity; presence of a minimum of 1.5-2mm of vertical thickness KM at the crest of alveolar mucosa in implant zone, Adequate amount of available bone for implant placement.

Exclusion criteria: Any history of metabolic or systemic disease affecting the integration of implant or connective tissue health surrounding implant, history of head or neck radistion, smokers, pregnant or lactating women, untreated generalized periodontitis, poor oral hygiene, psychiatric disorders, acute infection (abscess) at the intended site and Under treatment or had previous treatment with bisphosphonates.

**Surgical procedure**

Before commencing surgery, surgical site is examined clinically and radiographs were taken to get the brief idea of surrounding structures. During surgical procedure all 30 patients were prepared well-informed about flapless immediately loaded OPI and TPI protocols. Local anesthesia was induced by infiltration with lignocaine (2%) and adrenaline (1:80,000) for the both groups.

Group-(A) OPI:

- Patients were instructed to rinse preoperatively for 1min with 0.12% chlorhexidine solution, to reduce total mouth bacterial load,

- An initial pilot drill with select length were used for site preparation to give needlepoint accuracy for position, angle, and depth along with the use of copious saline irrigation. The drill should pass through the cortical bone and then the cancellous bone, and then shaping drill were used to prepare the exact bed size of the implant.

- Implant was then placed using a carrier and rotated clockwise and final implant position should carry out by using a torque rachet (torque 30-40Ncm). Primary stability was mandatory.

- The provisional acrylic resin restoration (temporary crown) was fabricated and care should be taken to
achieve a smooth contour to avoid soft tissue irritation, which was temporarily cemented out of occlusion for 2 months. The patients were instructed to avoid directly biting on the provisional restoration and to consume easily chewable food and maintain good oral hygiene. Following which, permanent restoration procedures, porcelain fused to metal (PFM) crown fabricated and cemented (figure1).

Group-(B) TPI:

- Same surgical procedure was performed for TPI but it was differed in loading protocol.

- Healing cap placed for gingival growth. The patients were instructed to maintain good oral hygiene.

- Two months after healing cap was removed, permanent restoration procedures were performed using silicone impression material (open tray technique). Finally, porcelain fused to metal (PFM) crown was fabricated and cemented (figure2).

Figure1: Group-A (OPI)– (a) Pre-operative; (b)Immediate post-operative; (c)Temporary prosthesis placed; (d)Final prosthesis after 2 two months; (e)Final prosthesis after six months

Figure2: Group-A (TPI) – (a)Pre-operative; (b,c)Intera-operative; (d)healing cover placed; (e)Abutment after two months; (f) final prosthesis after two months (g)Final prosthesis after six months.


**Evaluation of treatment outcome**

Patients were evaluated with the following clinical parameters at baseline (BL) two month after implant placement (definitive prosthesis placement) and six months follow up (6M).

A. **Width of keratinized mucosa (10)**

The width of the keratinized mucosa was measured at the mid-facial aspect of each implant using Williams probe. Each measurement was made from the gingival margin to the mucogingival junction. The mucogingival junction was identified by the rolling technique, in which the mucosa was rolled until the non-movable portion of the attached keratinized tissue was identified.

B. **Papilla preservative index (8)**

Dental papilla evaluated clinically using a papilla preservative index PPI described by Jemt (8).

0 will be assigned when no papilla was present;

1 when less than half of the papilla present measured from the reference line to contact point;

2 when at least half of the papilla was present but not all the way up to the contact point;

3 Papilla filled up the entire proximal space and was in good harmony with adjacent papillae;

4 when the papilla was hyperplasic.

C. **Plaque index (11)**

The oral hygiene status was evaluated by the presence or absence of visible plaque present at soft tissue margin.

Score 0: No plaque

Score 1: Plaque recognized by running a probe across marginal surface of implant

Score 2: Plaque can be seen by naked eye

Score 3: Abundance of soft matter within the gingival pocket, gingival margin and the adjacent tooth surface.

**Data management and statistical analysis**

The data were recorded on a specially designed questionnaire, collected and entered in computer via Microsoft Excel worksheet (Excel 2010) and then analyzed using appropriate data system which is called Statistical Package for Social Sciences (SPSS) version 25 and the results were compared between patients with different variables, with a statistical significance level of < 0.05. The results presented as rates, ratio, frequencies, percentages in tables and figures and analyzed using T-test and Chi square tests.

**Results**

A total of 30 patients enrolled in this study, fifteen per each group. The surgical intervention (implantation) failed in three cases of OPI group with a success of rate 80%, while all TPI cases ended with success (success rate 100%) though this difference was not statistically significant and p-value was 0.06.

The data of Table 1 show that there were no any statistically significant differences between OPI and TPI groups in regard to PPI both mesially and distally, PI and WHK measurements at baseline and after six months of follow up. T – test was performed to compare between the average and standard deviations of both groups and p – values were more than 0.05 in all conditions as the averages were close to each other.
Table 1: Comparison between OPI and TPI regarding different measures.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Groups</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPIm, baseline</td>
<td>OPI</td>
<td>12</td>
<td>2.41</td>
<td>0.51</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>TPI</td>
<td>15</td>
<td>2.46</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>PPId, baseline</td>
<td>OPI</td>
<td>12</td>
<td>2.16</td>
<td>0.71</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>TPI</td>
<td>15</td>
<td>2.20</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>PI, baseline</td>
<td>OPI</td>
<td>12</td>
<td>0.84</td>
<td>0.03</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>TPI</td>
<td>15</td>
<td>0.86</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>WKM, baseline</td>
<td>OPI</td>
<td>12</td>
<td>5</td>
<td>0.97</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>TPI</td>
<td>15</td>
<td>5.4</td>
<td>1.17</td>
<td></td>
</tr>
<tr>
<td>PPIm, six months</td>
<td>OPI</td>
<td>12</td>
<td>2.50</td>
<td>0.52</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>TPI</td>
<td>15</td>
<td>2.53</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>PPId, six months</td>
<td>OPI</td>
<td>12</td>
<td>2.50</td>
<td>0.52</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>TPI</td>
<td>15</td>
<td>2.67</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>PI, six months</td>
<td>OPI</td>
<td>12</td>
<td>0.58</td>
<td>0.66</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>TPI</td>
<td>15</td>
<td>0.60</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td>WKM, six months</td>
<td>OPI</td>
<td>12</td>
<td>4.83</td>
<td>0.83</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>TPI</td>
<td>15</td>
<td>5.23</td>
<td>1.01</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

The current study was performed to assess the clinical soft tissue outcomes of single-tooth implants, comparing immediately loaded OPI and early loaded TPI. All TPI were successfully integrated and in function for 6 months, leading to a 100% success rate. However, three of 15 immediately loaded OPI failed to osseointegrate, yielding 80% success rate (20% failure rate) and this result is near to previous study done by Oh et al. Previous studies reported a wide range of failure rates in immediately loaded single tooth implants, 0% to 19%. On the other hand, other studies reported no failures in immediately loaded single-tooth implants in the anterior maxilla. The differences in the failure rate between the studies might have resulted from several factors: different case selection criteria, time of functional loading (i.e., permanent crown placement) and poor bone quality. Another reason for failure in the IL group maybe due to excessive occlusal forces applied by patient.
Generally, the cause for papilla reduction after implant placement could be due to elevation of adjacent papilla during implant surgery. This was minimized by using a flapless approach in our study. The interdental papilla height slightly increased after six months of implant placement for both groups, which might have resulted from tissue remodeling after surgery and reformation of biological width. This is similar to a previous randomized controlled clinical trial by Oh et al.

The PI were recorded using the index described by Mombelli A et al. When the mean score between the groups were compared at different time points, there was no statistically significant difference. This is in accordance with other studies by Buser et al. and Oh et al. In general, the patients performed good home care and maintained good oral hygiene. Patients were informed about the subsequent consequences of plaque accumulation around implants and they were motivated to maintain oral hygiene by demonstrating oral hygiene techniques at every visit.

The WKM was measured at the mid-facial aspect of each implant using Williams probe. When the mean width of keratinized mucosa between group-A and group-B were compared at different time points, there was no statistically significant difference. This is in accordance with other studies by Oh et al. and Watzak et al. which showed results close to our findings.

The contemporary study has several limitations, including small sample size, short-term follow up, and relatively high failures in group-A. However, within the limitations of this study, it can be concluded that flapless implant surgery may provide esthetic soft tissue results in single-tooth implants either immediately loaded OPI or early loading TPI. More studies with larger sample size and long term follow up are recommended to verify the soft tissue outcomes of both groups.

**Conclusion**

From the results of this randomized clinical trial, we concluded that, after six months follow up there were no significant differences in implant esthetic between the immediately loaded OPI and early loading TPI treatment protocols regarding clinical parameters like PPI, WKM and PI.

**Conflict of Interest:** Not

**Ethical Clearance:** The study was approved by the Ethics Committee of the College of Dentistry, Hawler Medical University, Kurdistan Region, Irbil - Iraq.

**Source of Funding:** myself

**References**


Effect of CaCl$_2$ Crosslink Solution Concentration on the Characteristics of Gelatin Alginate Microspheres

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Abstract

Alginate as a constituent of microspheres matrix has a weakness, namely the lack of ability to slow the release of drugs from the system, causing the effects of drug therapy to the body to be less than optimal. To obtain preparations with slowed release, sodium alginate needs to be combined with other polymers such as gelatin. This study aims to determine the effect of the concentration of CaCl$_2$ connection solution on the characteristics of alginate-gelatin microspheres. In this study, alginate-gelatin microspheres were prepared using ionotropic gelation aerosolization techniques. Three formulas were made, each of which was replicated three times. F1 with CaCl$_2$ concentration 0.5 M, F2 with CaCl$_2$ concentration 1.0 M, F3 with a concentration of 1.5 M. The three formulas were made in the ratio of alginate-gelatin (1.5: 1)% and a crosslinking time of 1.5 hours. Evaluation of the characteristics of microspheres includes yield percentage, organoleptic, moisture content, morphology of microspheres, size and size distribution of microspheres, and swelling index. The data of this study were analyzed using one way ANNOVA. The results reveals a loss of specific uptake of the COO-manuronate alginate group and the occurrence of wave number shifts as well as an increase in the absorption intensity of the C = O group. Organoleptic examination results of alginate-gelatinin microspheres showed that microspheres of the three formulas had the same organoleptic, and formed spherical microspheres. On the inspection of swelling index, it was found that F1 and F2 reached the peak of swelling at 2 o’clock, while F3 reaches the peak of swelling at 3 o’clock. The greater the level of CaCl$_2$, then the average diameter of microspheres will also increase, while the swelling index will decrease with increasing levels of CaCl$_2$. In the results of moisture content examination, there is no difference in different CaCl$_2$.

Keywords: alginate, gelatin, microspheres, CaCl$_2$, ionotrophic gelation, aerosolization.

Introduction

Drug delivery system is a system designed to deliver active ingredients and provide therapeutic effects on the body $^1$. The drug delivery system aims to minimize the release of drugs in unwanted areas, avoid harmful side effects, and increase effectiveness $^2$. Delivery systems for topical preparations can be aimed to provide local or systemic effects. Some delivery systems used for topical preparations include liposomes, niosomes, solid lipid nanoparticles, and microspheres (Castro et al., 2008). Microsphere is a particle that has micro size and spherical shape. The particle size of microspheres ranges from 1 µm to 1000 µm $^3$.

Microspheres can be made using a variety of methods including emulsification, coacervation and ionotropic gelation $^4$. On the method of ionotropic gelation, the formation of microspheres is based on the ability of the polymer to form a hydrogel in the presence of divalent or polyvalent cations from a cross-linking solution. Based on the literature review, CaCl$_2$ concentration that is commonly used as a cross-linking solution is 0.5 M, 1 M and 1.5 M with a cross-
connect time of 1.5 hours. Alginate as a constituent of microspheres matrix has a weakness: it lacks the capability to slow the release of drugs from the system. To obtain preparations with slowed release, sodium alginate needs to be combined with other polymers, for example gelatin.

Increasing the concentration of the crosslinking solution will increase the number of bonds between the polymer and the crosslinking solution, so that the matrix will be more compact and lower the swelling index, thus the drug release from the system will be slower. However, increasing the concentration of the crosslinking solution will result in a microsphere with large particle size. This large particle size is undesirable in topical preparations since it can cause a sandy appearance when used. In addition, a small particle size is more desirable because it will produce a wider surface area when applied since the drug release will be more optimal.

Based on the description above, this study was conducted to determine the effect of CaCl₂ crosslinking solution (0.5; 1; 1.5) M on the characteristics of microspheres with sodium alginate-gelatin matrix (1.5: 1)% which includes percent recovery, moisture content, shape and surface, particle size, and swelling index. Microspheres production is done using the ionotropic gelation aerosolization techniques with a cross-connect time of 1.5 hours. The aim of this research is that the results are expected to be used as consideration in developing microspheres as delivery systems for topical routes with local effects.

Research Method

Research Material

This research uses Low Molecular Sodium Alginate (Pharmaceutical grade), Gelatin B (Pharmaceutical grade), Calcium Chloride (Pharmaceutical grade), Maltodextrin (Food grade), HCl 0.1 N, Aquadem. microspheres production is done using ionotropic gelation aerosolization techniques. Ionotropic gelation is a simple method, does not use high temperatures, does not use organic solvents and the use of aerosolization techniques can produce smaller and more uniform particle sizes.

Alginate-gelatin solution is sprayed into the CaCl₂ solution which have been made using atomizer with nozzle size of 200 μm, under constant pressure at a distance of 8 cm from the surface of the crosslinking solution and rotated at a speed of 1000 rpm for 1.5 hours. The process will produce a suspension of microspheres which are then filtered using a Buchner funnel and washed with aquadem until it is free of CaCl₂.

Research Tools

The tools used in this study are stirrer plate (Dragon Lab MS pro), pH meter SCHOTT glass mainz type CG 842, FTIR spectrophotometer (Jasco FT-IR / 5300), aerosolization spray (nozzle size 200 μm and 40 Psi pressure), analytical scales, ovens, petri dishes, optical microscopes, moisture analyzer and other glassware.

Variable Research

This study aims to determine the characteristics of alginate-gelatin microspheres with a ratio of 1.5%: 1% and CaCl₂ crosslinking solution with concentrations of 0.5 M, 1 M, 1.5 M, made using the ionotropic gelation aerosolization technique with a cross-connect time of 1.5 hours. The independent variable is the concentration of CaCl₂ crosslinking solution, and the dependent variable is the microspheres’ characteristics. While the control variables are the viscosity of the solution, the pH of the solution, the volume of CaCl₂ and polymer solution, concentration of polymer solution, ratio of alginate-gelatin concentration, diameter of nozzle, spray pressure, and the distance of the CaCl₂ solution with nozzle.

Analysis of Data

Examination of Moisture Content

Moisture content value is expressed in percent. This value is obtained from the numbers listed on the digital screen of moisture analyzer. Replication was done three times and the results are averaged. The moisture content obtained is compared with the conditions of moisture content from the references.

Morphological Examination of Microspheres

The shape and surface of the microspheres were observed based on the results obtained from Scanning Electro Microscope (SEM). This results in the form of three-dimensional appearance of the microspheres. This three-dimensional picture can be compared in terms of surface flatness and the spherical form of the microspheres produced from the three formulas made.
From the determined size of 300 particles, they are grouped into several intervals and classes. Then the particle diameter and percentage are determined. After that, the calculation results are presented in the form of a histogram with particle diameter as abscissa, and percent of particles as ordinate.

The average diameter of microspheres of each formula is calculated by the following formula:

\[
D_{\text{average}} = \frac{\sum d \cdot n}{\sum n}
\]

Note: \(n\) = number of microspheres observed \(d\) = microsphere size

From the calculation results, average diameter of microspheres was obtained, and the results were compared between formulas. Data analysis was done using One way ANOVA.

Examination of Swelling index

Swelling index is expressed as percent swelling (Ps) in the form of a graph with the x-axis in the form of time (hours) and the y-axis in the form of % swelling (weight change) at every hour. After obtaining the graph, the parameters to be compared between formulas are time and swelling profile from the microspheres produced.

Table 1 Average particle size of alginate-gelatin microspheres

<table>
<thead>
<tr>
<th>Formula</th>
<th>Replication</th>
<th>Particle size (µm)</th>
<th>Average ± SD</th>
<th>%KV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>2.84</td>
<td>2.82 ± 0.02</td>
<td>0.75%</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.83</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>3.60</td>
<td>3.51 ± 0.14</td>
<td>3.96%</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>4.23</td>
<td>3.90 ± 0.29</td>
<td>7.34%</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.75</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

From table 2 it can be seen that the particle size obtained already meets the recommended size as a microsphere intended for topical use, which is less than 300µm. Yield percent of the microspheres produced were analyzed statistically using the one way ANOVA method and tested post hoc with Fisher LSD using the IBM SPSS Statistics 22.0 program with a 95% confidence level. The particle size of microspheres in each formula are F1 = F1 = 2.82 ± 0.02; F2 = 3.51 ± 0.14; F3 = 3.90 ± 0.29 respectively.

Based on the results of statistical analysis, a value is obtained; \(\text{sig} = 0.001\). Value of \(\text{sig} <0.05\) indicates a significant difference in the yield percent from microspheres produced by all three formulas. After post hoc testing it can be seen that the difference is in F1 and F2 with values of \(\text{sig} = 0.004\); F2 and F3 with values of \(\text{sig} = 0.041\) and F1 and F3 with values of \(\text{sig} = 0.000\). This means that the difference in CaCl2 concentration has an effect on yield percent of microspheres. Examination of swelling index was done with three replications using the weighing method.
Table 2: Results of Alginate-Gelatin Microspheres Swelling (1.5: 1.0)%

<table>
<thead>
<tr>
<th>Formula</th>
<th>% Swelling Average ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st Hour</td>
</tr>
<tr>
<td>F1</td>
<td>378.67 ±11.37</td>
</tr>
<tr>
<td>F2</td>
<td>254.67 ±12.22</td>
</tr>
<tr>
<td>F3</td>
<td>171.33 ±41.68</td>
</tr>
</tbody>
</table>

From the table above it can be seen that the high levels of CaCl₂ (1.5 M) takes longer to reach the swelling peak. Based on the results of data processing of swelling index every hour, statistical analysis was carried out using one way ANOVA with a 95% confidence interval and the LSD Hoc post test.

Table 3 Data Statistics Test Results of Alginate-Gelatin Microspheres Swelling (1.5: 1.0)%

<table>
<thead>
<tr>
<th>Swelling Percent/ Hour</th>
<th>Significant Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1 to F2</td>
</tr>
<tr>
<td>1</td>
<td>0.001*</td>
</tr>
<tr>
<td>2</td>
<td>0.790</td>
</tr>
<tr>
<td>3</td>
<td>0.151</td>
</tr>
<tr>
<td>4</td>
<td>0.710</td>
</tr>
<tr>
<td>5</td>
<td>0.021*</td>
</tr>
<tr>
<td>6</td>
<td>0.208</td>
</tr>
</tbody>
</table>

*: there are significant differences in the swelling value between the two formulas.
Discussion

Based on post hoc test result, it can be seen that there are differences in F1 and F2 with values of \( \text{sig} = 0.001 \); F2 and F3 with values of \( \text{sig} = 0.031 \) and F1 and F3 with values of \( \text{sig} = 0.021 \). According to a literature study, percent recovery should increase with an increase in \( \text{CaCl}_2 \) level, as long as polymer is still available to form bonds. However, in this study the highest percentage of recovery was obtained in F2 with \( \text{CaCl}_2 \) levels of 1.0 billion and decreased in \( \text{CaCl}_2 \) levels of 1.5 M. This can be caused by several factors including the calculation of percent recovery which is influenced by the addition of maltodextrin and the drying process using freeze drying which cannot be controlled by researchers.

Tables above show that the morphology of alginate-gelatin microspheres using SEM, it was found that the resulting microspheres were spherical in shape and had a flat surface. This can be seen in Figure 5.4. Whereas the particle size examination obtained particle size for F1 = 2.82 ± 0.02; F2 = 3.51 ± 0.14; F3 = 3.90 ± 0.29 respectively. Based on the results of statistical analysis, a value is obtained; \( \text{sig} = 0.001 \). Value of \( \text{sig} < 0.05 \) showed a significant difference in particle size of alginate-gelatin microspheres. Based on the post hoc test result it can be seen that the difference is in F1 and F2 with values of \( \text{sig} = 0.004 \); F2 and F3 with values of \( \text{sig} = 0.041 \) and F1 and F3 with values of \( \text{sig} = 0.000 \) (Liu & Wang, 2011).

The results of the qualitative analysis of sodium alginate organoleptically shows data in accordance with the research hypothesis. In the analysis with FTIR there are some specific uptake, namely uptake at 1599.76 cm\(^{-1}\) and 1405.42 cm\(^{-1}\) which shows the presence of carboxylic groups and uptake at 815.11 cm\(^{-1}\) which is the fingerprint area of manuronates. In addition, the results of observations of thermal analysis using DTA found a peak in the region of 233° C, this is in accordance with the data in the literature which states that on the sodium alginate thermogram peak will occur in the area of 191.15-254.6° C. From these results it can be seen that the difference in \( \text{CaCl}_2 \) level has no effect on organoleptic alginate-gelatin microspheres.

The results of organoleptic examination of maltodextrin showed that the maltodextrin used was in accordance with the data in the references, which is in the form of fine white powder, has a typical odor, and not sweet. The results of the analysis with FTIR show that there are some specific uptake that is consistent with the data in the references. Based on the references, maltodextrin will provide absorption at wave numbers between 980-1200 cm\(^{-1}\) which shows the presence of CO groups and absorption at 3400 cm wave number which indicates the presence of C = O carboxylic groups. In the results of the examination of gelatin used, an uptake was found at wave value of approximately 1021.5 and 1080 cm\(^{-1}\) which is a CO group. Uptake at around 1157.7 cm\(^{-1}\) and 3429.6 cm\(^{-1}\) which is the C=O group.

According to Mi et al. in his research (2019), microspheres as a delivery system for topical routes are expected to have a small size and homogeneous and can release the drug slowly. This is related to the acceptability and effectiveness of topical preparations. Preparations with slow release can be obtained from microspheres with algin-gelatin combination matrices using ionotropic gelation method with a \( \text{CaCl}_2 \) crosslinking solution. Sustained release in alginate-gelatin microspheres occur due to the interaction between Ca ions with the COO group guluronate in alginites and NH groups gelatin with the COO group manuronate in alginites, causing the microspheres formed to have a more compact structure.

The achievement of topical preparations with the desired criteria is influenced by the characteristics of alginate-gelatin microspheres which include morphology, size and size distribution and swelling index. The characteristics of the microspheres are influenced by several factors, including the concentration of the polymer, the cross-linking time and the concentration of the cross-linking solution. The focus of this research is to investigate the effect of different levels of \( \text{CaCl}_2 \) crosslinking on the characteristics of alginate-gelatin microspheres prepared using the ionotropic gelation aerosolization techniques. The characteristics of microspheres compared in this case include, percent recovery, size and size distribution and swelling profile.

The efficiency of the microsphering process can be seen from the recovery obtained in each formula. The results of the calculation of percent recovery are F1 = 39.77 ± 3.30; F2 = 64.69 ± 7.72; and F3 = 52.85 ± 2.72. From these results a statistical analysis was conducted using one way ANOVA and obtained the value of \( \text{sig} = 0.003 \). A value of \( \text{sig} < 0.05 \) indicates that there is a significant difference in the percent recovery results with a difference in \( \text{CaCl}_2 \) levels.
Observation on swelling index was done to seek the profile or pattern of swelling from each formula to estimate the pattern of drug release from the microsphere formula. Based on the observations it is obtained that each formula has a different swelling profile. F1 and F2 reached the swelling peak at the same hour, which is the second hour, but both have different peak swelling values. F1 reaches greater top swelling compared to F2. The peak swelling value of the two formulas in sequence are F1 = 469.33 ± 15.14, F2 = 410.67 ± 55.08.

Conclusion

Based on the results of the study, it can be concluded that an increase in CaCl2 (0.5; 1.0; 1.5)M levels in alginate-gelatin microspheres (1.5:1) had no effect on organoleptic and morphological microspheres, yet it increases microsphere particle size. Based on the observations it is obtained that each formula has a different swelling profile. F1 and F2 reached the swelling peak at the same hour, which is the second hour, but both have different peak swelling values.

Statement of Informed Consent

This study involves participants in the process using a questionnaire that was accordant with the ethical research principle based on the regulation of research ethic committee. The present study is carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, non-maleficence, and justice.

Conflict of Interest: There is no report about conflict of interest since this study has been conducted until now.

Source of Funding: All of the research processes is funded by the authors only.

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References


Association of Adiponectin and IgE levels with occurrence of asthma in Babylon Province

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Abstract

Background: Asthma is a health condition that affects breathing in people. Breathing comfortably is not something most of us need to think about. But if you have asthma, there are times when you have difficulty breathing. The main objectives in the current study are: Determine the role of adiponectin, a hormone that acts as an anti-inflammatory, In addition determine the concentration of immunoglobulin E (IgE) In asthma patients.

Method: This study included two groups: Group 1: 80 Patients with asthma were divided into two parts (40 samples obese and 40 samples non-obese). Group 2: 80 Group of healthy subjects with no history or clinical evidence of asthma. samples were divided into two parts (40 samples were obese and 40 were not obese). Asthma was diagnosed through a pulmonary function test (PFT) and then invited to a private clinic and measured nitric oxide gas (FeNO) to confirm the diagnosis. Enzyme-linked immunosorbent assay (ELISA) technique was used to determine the concentration of adiponectin and IgE in serum.

Results: The present study demonstrated the increase of adiponectin level with asthmatic patients compared with the control group and showed significant difference between non-obese patients group and non-obese healthy group.

Conclusion: Adiponectin related with obesity play crucial role in asthma development and inverse relationship between IgE and adiponectin.

Key words: Adiponectin, IgE, ELISA, BIM, FeNO

Introduction

Asthma is a disease of reversible airflow obstruction, bronchial hyper responsiveness, and underlying airway inflammation. In other words, it is a disease appear by effect allergic factors and non-allergic factors and result in bronchial obstruction and inflammation so it is multifactorial disease. This disease ranked 16th among the leading causes of years lived with disability and 28th among the leading causes of burden of disease. There are many reasons for asthma, but almost certainly reflect variable contributions of genetic and environmental factors in different regions. So Immunoglobulin E (IgE) is the antibody responsible for allergic reactions and is important to the pathogenesis of allergic diseases and the evolution and persistence of inflammation. IgE attaches to cell surfaces and a specific high-affinity receptor. Furthermore, there are some substance like adiponectin, it also seems influenced to the asthma. Adiponectin is a 244 amino-acid-long polypeptide. The adiponectin has four distinct regions. There are many studies on the effect of levels adiponectin on asthma. However, human data remain inconclusive and not known if may be the adiponectin affects asthma control by treatment. Shore in their study of weak mice suggests that exogenous dose from the adiponectin relieve allergen-induced airway hyperreactivity and eosinophilic influx in both sexes. Aims of this study that estimate role adiponectin in pathogenesis of asthma, estimate role IgE in pathogenesis of asthma and to find possible association between IgE with adiponectin.

Method

at the laboratory of Biochemistry Department. This study was complete some process in a College
of Medicine, University of Babylon at the laboratory of Biochemistry Department. The subjects in this prospective case-control research, include a total of 160 subjects 80 asthmatic subjects (42 females with 38 males) and 80 clinically healthy control subjects (37 females with 43 males). All samples were collected during the period from the August - 2018 till February - 2019. Patients were collected from Marjan Hospital and invited to a specialized clinic for examination by fractional exhaled nitric oxide (FeNO) device. Asthmatic subjects were categorized to (40 obese asthmatic subjects with BMI mean SE 34.5 0.52 and 40 non-obese asthmatic subjects with BMI mean 25.40.45), Also control group subdivided into two groups (40 obese control subjects with BMI mean33.7 0.56 and 40 non-obese control subjects 25.050.45). with BMI mean.

Inclusion Criteria: The inclusion criteria for the selection of the study subjects include, Asthmatic patients group (depending on pulmomologist decision according to the GINA guideline). All person accepter to participate in the current study. Age of all subjects was ≥18 years old.

Exclusion Criteria: Any person suffered from the following were excluded from this study: Patient with diabetes. Heart diseases, hypertension, malignancy, and any chronic disease. Patient with any other chronic disease (metabolic and endocrine)Smokers. Pregnancy women. Patient with chronic obstructive pulmonary disease (COPD) disease and emphysema. Another lung disease.

Body Mass Index (BMI):

Body mass index (BMI) is the ratio of a person’s weight to height in square meters; it commonly used to classify weight as healthy person or unhealthy person. BMI calculated by below equation\(^1\) as follow:

\[
BMI(Kg/m^2) = \frac{\text{Weight (Kg)}}{(\text{Height})^2 \text{ m}^2}
\]

Table 1: Demographic characteristics of the study groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study groups</th>
<th>No.</th>
<th>Means ±SE</th>
<th>P- value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Asthmatic patients</td>
<td>80</td>
<td>34.06 ±1.28</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>80</td>
<td>33.45 ±1.27</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>Asthmatic patients</td>
<td>80</td>
<td>30.77 ±0.61</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>80</td>
<td>28.66 ±0.65</td>
<td></td>
</tr>
<tr>
<td>Waist/ Hip ratio</td>
<td>Asthmatic patients</td>
<td>80</td>
<td>0.89 ±0.01</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>80</td>
<td>0.89 ±0.008</td>
<td></td>
</tr>
</tbody>
</table>

P value 0.05 was significant.
BMI (body mass index)

Age:

The results were expressed as means and standard error (SE). There was no significant difference (p > 0.05) in age (as means) between control and asthmatic patients, means ±SE for the case is (34.06 ±1.28) and for control (33.45 ±1.27) as appear in Table 1. When we matching the age this is play a role to eliminate differences in results that may be due to the big variation in age 15.

Body mass index (BMI):

There was significant difference (p < 0.05) in Body mass index (BMI) (as means) between control groups and asthmatic patients, mean ±SE for the case are (30.77±0.61) and for control (28.66±0.65) as shown in table 1-1. Even though we were already taken in both groups obese and non-obese. So these results support some of the studies indicated a significant difference in the overlap between asthma and obesity, with suggesting that the association of obesity with both incidence asthma and prevalence it in women more than in men 16. So, the difference in BMI between patients and controls in this study accepted with the other studies 17,18.

Waist to Hip Ratio:

There was no significant difference (p > 0.05) in waist/hip ratio (as means) between control and asthmatic patients, means ±SE for case is (0.89 ±0.01) and for control (0.89 ±0.008), Table 1-1. Although there are many studies showed that BMI was associated with asthma as risk factor 19 but, waist/hip ratio shown independent factor than BMI 11.

Gender:

Other factor effect on the asthma it is gender.

The Figure above indicates a slight difference between male and female, and some studies can be adopted like Zein and Erzurum that shown asthma is severing and more prevalent in women especially with multiple gestation or with early menarche, offer a role for sex hormones on asthma disease 20.

Biochemical Result:

Mean differences of adiponectin level according to studied groups:

Mean differences of biochemical results including adiponectin was revealed in Table 2. The significant difference (p < 0.05) in adiponectin (as means) between control groups and asthmatic patients, means ±SE for the case is (140.50±8.46) and for control (114.27 ±7.48). The results were shown in the Table 1-2 and the concentration of adiponectin for asthmatic patients was higher than the control group.

Table 2 Mean difference SE for Adiponectin level.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study Group</th>
<th>No.</th>
<th>MeanSE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin</td>
<td>Asthmatic patients</td>
<td>80</td>
<td>140.50±8.46</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>80</td>
<td>114.27±7.48</td>
<td></td>
</tr>
</tbody>
</table>

P-value 0.05 was significant.

Adiponectin is a protein hormone and is 30 kDa in size; In adults it is excrete and synthesized solely by adipose cells, the levels of adiponectin in plasma are inversely proportional to percentage of fat. A little levels of adiponectin shown in Obese individuals 21. With higher adiponectin levels in animal models leads to minus airway responsiveness and inflammation as a challenge for allergens 22. Furthermore, this hormone prevents the proliferation of cultured vascular smooth muscle cells 23. Sood et al. showed that higher serum adiponectin concentrations were independently associated with adverse clinical outcomes of asthma (such as asthma-related symptoms, medications and disease activity) among men but not women 24.
Yet, poor muscle function and physical disability are linked with higher levels of adiponectin in circulating blood, which suggests a possible related between risk of falls and adiponectin.

Mean differences of IgE level according to asthmatic patient group:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study group</th>
<th>NO.</th>
<th>MeanSE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgE</td>
<td>Non-allergic</td>
<td>43</td>
<td>80.089.22</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Allergic</td>
<td>37</td>
<td>601.00 43.46</td>
<td></td>
</tr>
</tbody>
</table>

Some studies have reported the increased levels of IgE in asthmatic. Thus, it is in conformity with the well-known fact that IgE plays a major role in the pathophysiology of asthma and another allergic disorder.

Correlation between serum adiponectin and IgE in asthmatic patients:

The result in table 4 appeared no significant difference between the adiponectin and IgE statistically but there is an inverse relationship between them.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adiponectin</th>
<th>IgE</th>
</tr>
</thead>
<tbody>
<tr>
<td>adiponectin</td>
<td>Pearson correlation</td>
<td>Sig. (2-tailed)</td>
</tr>
<tr>
<td>IgE</td>
<td>Pearson correlation</td>
<td>Sig. (2-tailed)</td>
</tr>
</tbody>
</table>

Serum adiponectin in (obese and non-obese) for patient and control Group.

Table 5 revealed that mean SE of adiponectin in asthmatic obese are (119.2510.70) and control obese are (1068.21) respectively so no significant difference. While significant differences between patient non-obese and control non-obese P-value <0.05 another compares between patient obese and non-obese and appeared no significant difference also.

<table>
<thead>
<tr>
<th>Type</th>
<th>No.</th>
<th>meanSE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient obese</td>
<td>40</td>
<td>119.25±10.70</td>
<td>0.21</td>
</tr>
<tr>
<td>Control obese</td>
<td>40</td>
<td>106.57±8.21</td>
<td></td>
</tr>
<tr>
<td>Patient non-obese</td>
<td>40</td>
<td>171.16±11.21</td>
<td>0.01</td>
</tr>
<tr>
<td>Control non-obese</td>
<td>40</td>
<td>108.73±7.96</td>
<td></td>
</tr>
<tr>
<td>Patient obese</td>
<td>40</td>
<td>119.32±10.70</td>
<td>0.36</td>
</tr>
<tr>
<td>Patient non-obese</td>
<td>40</td>
<td>154.09±11.85</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

The reduction in adiponectin in obese persons possible participate to rise smooth muscle mass in asthmatic persons 29. But in this study the level of adiponectin in asthmatic patient was high so the most common risk factor for falls is muscle weakness 30. The change of the pattern in asthma therapy perhaps include weight regulation and may be change pharmacotherapeutic systems shall require special interest to that underserved populations face in the treatment of asthma. These obstructions include limited access to care and medications, cultural changes in understanding and accepting treatment options, and decreased health literacy 31,32. To manage these complexities, innovative way to therapy are needed. There is actual evidence that obesity and asthma are linked together 33.

Ethical Clearance: Approval by scientific committee of Babylon Medical College (University of Babylon, Iraq) and the Biochemistry Department in the medical college.

Source of Funding: The funding body written out in full by self

Conflict of Interest: If any then mention it otherwise write it as nil

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Comparison of the Total amount of Macrophages on Full Thickness Wound Bed in The Use of Tulle, Freeze-Dried Amnion, and Microbial Cellulose

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Abstract

Background: The wound is the most frequent problems faced by plastic surgeon. Many factors can affect the wound healing process. Macrophages are as one indicator of wound healing which present in the wound within 24-48 hours after injury. Currently, there is a variety of wound dressing available which can increase the levels of macrophages in the wound healing process according to previous research.

Objectives: To compare the total amount of macrophages on full thickness wound bed in the use of tulle, freeze-dried amnion, and microbial cellulose on the second day.

Material and Method: The study design was experimental, post-test only group design using 21 male rats Rattus norvegicus. The wound was closed with tulle, amnion, and microbial cellulose which was evaluated on the second day. The samples wounds were fixed by 10% formalin solution then examination of samples was conducted by Wright-Giemsa staining routine/Hemato-eosin under a microscope.

Results: Macrophages obtained at a given tulle ranges between 41-96, freeze-dried amniotic at 51-142, and the microbial cellulose at 55-96. In other hand, the mean number of macrophages in the wound by the microbial cellulose at 77.4; its 1.3 times higher than the given tulle at 59; meanwhile its 0.9 times lower than the freeze-dried amniotic given by 83.

Conclusions: There were no differences in the increase number of macrophages in the wound bed by the use of tulle, freeze-dried amnion and microbial cellulose on the second day.

Keywords: Amnion, macrophage, microbial cellulose, tulle, wound healing

Introduction

Although the etiology of an injury could be different, the healing process still remains the same. Damage to the tissue will stimulate the activation of extrinsic and intrinsic factors, acute and chronic inflammatory responses, the process of neovascularization through angiogenesis and vasculogenesis, cell proliferation, mitosis, apoptosis, and extracellular matrix deposit and remodel the matrix.

In addition to the intracellular processes, wound healing could be affected by the condition of the wound. The condition of the wound made in such way to create the appropriate atmosphere to accelerate the wound healing process. Macrophages are very important in wound healing process since they are capable of releasing cytokines and materials. They are needed to help in the healing process of wound. Macrophages present in the wound within 24-48 hours after injury...
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1923

and peaked at 48-72 hours. Moreover, macrophages are capable of producing growth factors, such as TGF-β and an epidermal growth factor that work in regulating the inflammatory response, stimulate angiogenesis, and granulation tissue formation realigned 1,3.

Macrophages play an important role as the main source of most of the active ingredient in the process of wound healing. The majority of the necessary growth factors in wound healing are produced by macrophages. Macrophages are required in the process of wound healing due to the removal of macrophages. If there is no macrophage, so it will cause wound healing to stop 4,5.

The process of wound healing occurs in three phases, namely the inflammatory phase, proliferation phase, and remodeling phase. After the injury, blood vessels constrict and retraction broke with hemostasis reaction. Furthermore, the release of histamine from mast cells that also works to increase vasodilation and capillary permeability. Inflammatory phase is characterized by increasing of vascular permeability, especially for leukocytes, neutrophils, and macrophages 1,6.

Currently, there are a variety of dressings available which according to research can increase the levels of macrophages in the wound healing process. In this study, researchers tried to observe the local response in the wound bed with tulle applications, freeze-dried amnion, and microbial cellulose associated with macrophages as one of the main components in the wound healing. It is expected that the results of the research can be used as the development of the use of freeze-dried amnion and microbial cellulose.

**Materials and Method**

This research uses experimental study design, post-test only group design. Operational variables consist of microbial cellulose, freeze-dried amnion, and tulle 7. We use Cuticell® epigraft as microbial cellulose, is a layer of pure cellulose derived from Acetobacter xylinum bacteria that has a great potential in wound healing. Microbial cellulose is proven to increase the rate of wound epithelialization. We used freeze-dried amnion which has gone through the process of freeze-dried and sterilization with gamma rays from Tissue Bank of Dr. Soetomo Hospital. Meanwhile, we used Cuticell® Classic as a tulle without any other additional ingredients (antibiotics).

Three months old of 21 mice (Rattus novergicus) were selected in this study which was randomized using providing labels. Mice were grouped into three groups consist of seven mice; tulle is applied in group 1, freeze-dried amnion is applied in group 2, while microbial cellulose is applied in group 3. Mice injected with ketamine 20mg/kg intra-muscular. Each mouse was purposely being cut in 1x1cm square-shaped size on their backs that will be disinfected with Betadine 10% and savlon 1: 30. Operation field was narrowed by dock sterile then a full-thickness wound was made by tangential excision using blade number 15. Injury in the group 1 covered with tulle, wound in the group 2 covered by the freeze-dried amnion, while in the group 3, the wound covered with microbial cellulose. After that, the wound was treated in a closed wearing thick sterile gauze and covered by stitches on the back. All mice were given the intramuscular injection of Penicillin Procaine 100mg/kg. Mice kept in different cages and fed by the same type and amount.

The wound was evaluated on the second day, where ten mice were sacrificed by injecting phenobarbital 60-100 mg/kg intraperitoneal on lateral midline area between processes hypoid and pubis. The second day was cones because macrophages infiltration in the wound bed started at 48-72 hours post-injury 2. Studies of macrophage activation showed that to look at the ability of macrophage activation should use the fastest time limit 4. Based on the data, the authors chose the fastest time which was the second day after the injury. On histologic examination, the wound dimensions of approximately 0.5 cm outside the wound edges was included as healthy tissue. Then, the tissue was removed until the muscle layer that will be folded with filter paper and fixed with 10% formalin solution. Routine examination was performed by Wright-Giemsa staining/ Hematokilin-eosin, then viewed under a microscope to count the number of macrophages with the help of graticule lens (ischak wirjatmadi).

**Results**

Normality test using the Kolmogorov-Smirnov test was performed on the number of macrophages before being tested statistically. The result of normality test for the three groups showed p value >0,05 which means all data were normally distributed.
Figure 1. The number of macrophages in the healing tissue. Macrophage cells (arrows) on the injury area with treatment using tulle (HE staining. Magnification 100x; H600L Nikon microscope; Fi2 300 Megapixel Camera DS).

Figure 2. The number of macrophages in the healing tissue. Macrophage cells (arrows) on the injury area with treatment using freeze-dried amnion (HE staining. Magnification 100x; H600L Nikon microscope; Fi2 300 Megapixel Camera DS).
Table 1. The amount of macrophages cell in each treatment

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mean</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tulle</td>
<td>59.9</td>
<td>24.7</td>
<td>41</td>
<td>96</td>
</tr>
<tr>
<td>Freeze-dried amnion</td>
<td>83.0</td>
<td>32.9</td>
<td>51</td>
<td>142</td>
</tr>
<tr>
<td>Microbial cellulose</td>
<td>77.4</td>
<td>15.2</td>
<td>55</td>
<td>96</td>
</tr>
</tbody>
</table>

It can be seen in figure 1, 2 and 3 that the number of macrophages in the healing tissue was observed histologically using 100x magnification. The calculation of macrophages was obtained by the treatment result using tulle that was about 599 by the maximum amount of 96 macrophages cells. The treatment of microbial cellulose showed the mean of 77.4 by the maximum cell of macrophages as same as tulle treatment which was about 96 cells. After that, the treatment used freeze-dried amnion resulted mean of 83.0 by maximum amount of macrophages cell was 142. Therefore, the treatment used freeze-drive amnion resulted effectively.

However, the standard deviation (SD) did not show the difference significantly and can be seen in figure 4. Macrophage of scar tissue is obtained at a certain range, for tulle at 41-96, freeze-dried amniotic at 51-142 and the microbial cellulose at 55-96.
It can be seen in table 1 that the mean number of macrophages on the wound by the microbial cellulose was 77.4 which means that it was 1.3 times higher than the tulle at 59; meanwhile it was 0.9 times lower than the freeze-dried amniotic at 83. Statistical test results obtained using ANOVA with p value >0.05 which means that there was no significant difference in the number of macrophages in the provision of microbial cellulose, tulle, and freeze-dried amniotic.

**Discussion**

In the previous studies stated that the amnion has an impact on the local immune system by giving more macrophage expression and regulate the function of macrophages 8,9. In addition, the amnion gives more influence number of macrophages in bed sores than tulle. The amnion increases the number of macrophages 2.5 times more than tulle. Microbial cellulose dressing may accelerate epithelialization while bacterial cellulose can regulate inflammation by giving effects on the formation of macrophages and lymphocytes 10,11. Those studies are different with the results of this research.

The macrophage acts as an indicator of the wound healing process that is absolutely necessary for the wound healing process. Macrophages are needed in the wound healing since they are capable of producing cytokines and growth factors such as TGF-β and the epidermal growth factor that works in regulating the inflammatory response, stimulate angiogenesis, and granulation tissue formation realigned. Macrophages present in the wound within 24-48 hours after the injury and peaked at 48-72 hours.

Other studies claimed that the use of amnion on wound gives less significant result in influencing the inflammatory phase of the healing process. Despite the significant increase seen in the process of angiogenesis in the granulation phase, it does not affect the process epithelialization, fibroplasia or fibrosis in phase significantly 12.

One of the factors that play a role and always used in the wound treatment is wound dressings (bandages). The amnion contains mesenchymal stem cells and growth factors that can accelerate the healing of a wound 13. The amnion effects on the local immune system by giving more macrophage expression and regulate the function of macrophages 8,9. Microbial cellulose dressing can accelerate epithelialization rate 10. Bacterial cellulose can regulate inflammation that effects on the formation of macrophage and lymphocyte 11,14. Bacterial cellulose stimulates macrophages to produce IL-12, p40, and TNF-α which play a role in anti-inflammatory and antigenic processes 15. This study proves that there is no difference in the number of macrophages in the wound bed given tulle, freeze-dried amnion, and microbial
cellulose.

Conclusions

There was no difference in the number of macrophages in the wound bed given tulle and microbial cellulose, given freeze-dried amnion and microbial cellulose on the second day.

Ethical Clearance: This study protocol was approved by ethical clearance Dr. Soetomo Teaching Hospital Surabaya, Indonesia.

Conflict of Interest: The author reports no conflict of interest of this work.

Source of Funding: This study is done with individual funding.

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Evaluation of Women’s Perception toward Childbirth Experience after Utilization of Pain Management Practices at Al-Elwyia Maternity Teaching Hospital

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¹Instructor, ²Professor, Maternal and Neonate Nursing Department, College of Nursing/ University of Baghdad

Abstract

Objectives: To evaluate the women’s perception with Childbirth Experience after utilization Pain Management Practices.

Methodology: A quasi-experimental study conducted on non-probability of (30) women whom admitted to Al-Elwyia Maternity Teaching Hospital suffering from labor pain for the period of (4th July 2018 through 24th October 2018). Descriptive & Inferential statistical analyses were used to analyze the data.

Results: show that the highest percentages of non-pharmacological methods used was frankincense oil, and related to women perception of labor pain they are assessed high as general, and they are accounted 24(96.0%).

Conclusions: The study concluded that there are a positive evaluation observed regarding women’s perception with childbirth experience after utilization pain management practices.

Recommendations: The study recommended developing structured training program and clinical practice guideline of non-pharmacological pain management methods during labor and childbirth should be made available in English and Arabic which contains information about the proper use of non-pharmacological pain management methods and its advantages to midwives working in delivery room.

Keywords: Assessment, Perception, childbirth, Pain Management.

Introduction

Labor pain is considered one of the most painful conditions in women life and these women often underestimate the pain they will experience due to several factors might influence a woman’s perception of labor pain. These factors include (cultural, ethnic, educational factors, excessive anxiety triggers “fight-or-flight” response, fear of pain may be one component of labor-related anxiety and has a high correlation with pain levels reported during first-stage labor. The environment affects the woman’s experience of pain that includes the persons present and their verbal and nonverbal communications, the philosophy of care and practice policies of the providers; the quality of support the woman perceives from those present, the degree of strangeness of the environment, including (furniture, noise, lighting, and temperature), Ability to cope all affects her ability to manage the pain of labor. (1-3)

Methodology

A quasi-experimental study design was conducted on purposive sample, of (30) women whom admitted to Al-Elwyia Maternity Teaching Hospital suffering from labor pain. Study implemented for the period of (4th July 2018 through 24th October 2018). Data collection will be gathered by application one of non-pharmacological strategies of pain relief methods include: (frankincense, jasmine, & olive oils), massage, body movement and change position (squatting, side-lying, & standing), breathing technique, and therapeutic touch) and by used questionnaire format which consisted of two parts, including non-pharmacological methods the delivering woman received during labor and delivered women’s perception toward labor pain which consisted of (25 items). A pilot study was carried out between the 25th June 2018, to 1st July 2018, on (10) women to determine the reliability of the questionnaire and content validity was carried out through the 12 experts. Descriptive and inferential statistical analyses were used to analyze the data.
Results

Figure (1) Cluster Bar Chart for the distribution types of non-pharmacological methods

Figure (1) results show that “Frankincense Oil” method has recorded the high and first ordered method, and accounted (30%), then followed with Jasmine Oil” method, and accounted (23.3%), then followed with “Squatting Position, and Side-Lying Position & Breathing” methods, and accounted (13.3%), Massage, Massage & Standing position, Therapeutic touch & Standing, Olive oil, breathing technique, & side lying position accounted (3.3%).

Table (1): Delivered Women’s Perception of their Labor Pain

<table>
<thead>
<tr>
<th>No.</th>
<th>Items</th>
<th>MS</th>
<th>Ass.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I was able to participate in making decision about how to manage labor pain</td>
<td>3.630</td>
<td>H</td>
</tr>
<tr>
<td>2</td>
<td>I was in control of my pain management</td>
<td>3.470</td>
<td>H</td>
</tr>
<tr>
<td>3</td>
<td>I was able to choose the type of pain management I would receive</td>
<td>3.570</td>
<td>H</td>
</tr>
<tr>
<td>4</td>
<td>The nurse asked my opinion about unplanned procedure before it was performed</td>
<td>3.600</td>
<td>H</td>
</tr>
<tr>
<td>5</td>
<td>I was able to move around as best I could even though I had some pain interventions</td>
<td>3.600</td>
<td>H</td>
</tr>
<tr>
<td>6</td>
<td>While I was in labor, I was able to decide how to be most comfortable.</td>
<td>3.530</td>
<td>H</td>
</tr>
<tr>
<td>7</td>
<td>I was able to take charge of managing my labor and birth</td>
<td>3.370</td>
<td>H</td>
</tr>
<tr>
<td>8</td>
<td>I was able to control the labor and delivery environment</td>
<td>3.170</td>
<td>H</td>
</tr>
<tr>
<td>9</td>
<td>Pain management practice helped me to calm down and not feel so tense</td>
<td>3.570</td>
<td>H</td>
</tr>
<tr>
<td>10</td>
<td>I feel comforted by the nurse when apply pain management practice</td>
<td>3.800</td>
<td>H</td>
</tr>
<tr>
<td>11</td>
<td>Therapies helped me feel relaxed</td>
<td>3.670</td>
<td>H</td>
</tr>
<tr>
<td>12</td>
<td>Presence of the nurse made me comfortable even though I was in pain</td>
<td>3.900</td>
<td>H</td>
</tr>
<tr>
<td>13</td>
<td>I enjoyed the way nurse communicated with me in clam manner</td>
<td>3.870</td>
<td>H</td>
</tr>
<tr>
<td>14</td>
<td>The nurse listened to me even when I was not making sense of the things I said</td>
<td>3.870</td>
<td>H</td>
</tr>
</tbody>
</table>
Table (1) shows that delivered women’s perception of their labor pain items are high as assessed generally, and they are accounted 24(96.0%), as well as only item named “I felt the nurse was more interested in her job than my needs” has a moderate assessment.

**Discussion**

**Non pharmacological methods**

The results of figure (1) show that aromatherapy are the first order used which include” frankincense oil, jasmine oil, and olive oil” then body movement and change position such as squatting position, side-lying position which used combination with breathing and recorded the second order and finally, massage, massage and standing position, therapeutic touch and standing, breathing technique, and therapeutic touch and distraction” are used in a little. In this study, it has been noted that essential oils (Frankincense, jasmine, and olive) recorded the high number of uses and accounted 17 (56.7%). The researcher attempted to use aromatherapy for participant (according to their preference) due to that the researcher noted the uses of these aromatherapy are widely in world and have many benefit such as promoting general relaxation, reduce anxiety and helpful to reduce labor pain as it used in the current study depend on wide evidence based studies\(^{(4, 5-7)}\). Relative to use of massage technique the results show that there are two women were choosing massage. The massage work as a form of pain relief by increasing the production of endorphins in the body that reduce the transmission of signals between nerve cells and thus lower the perception of pain \(^{(10)}\). Relative to use of therapeutic touch observed that there are two women chose therapeutic touch but in different way one therapeutic touch with standing position and another one use therapeutic touch with side-lying position.

**Women’s Perception of their Labor Pain Regarding Study sample**

According to the table (1), the results show that delivered women’s perception of their labor pain items concerning study group assigned that observed responses are high assessed generally, and they are accounted 24 (96.0%), as well as only item “I felt the nurse was more interested in her job than my needs” has a moderate assessed. These high assessed responses related to pivotal role that the researcher acted in care for women by using pain management practices in addition to her role as a doula (continuous labor support) in labor for all participants in study group. And these results are in agreement with evidence based studies from high

<table>
<thead>
<tr>
<th>Item</th>
<th>Score</th>
<th>Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 The nurse were really gentle when they were carrying out the practice</td>
<td>3.900</td>
<td>H</td>
</tr>
<tr>
<td>16 The nurse were of help to me in coping with labor pain</td>
<td>3.870</td>
<td>H</td>
</tr>
<tr>
<td>17 The nurse really encouraged me</td>
<td>3.900</td>
<td>H</td>
</tr>
<tr>
<td>18 I felt free to talk to this nurse about what concerned me</td>
<td>3.870</td>
<td>H</td>
</tr>
<tr>
<td>19 I felt the nurse was more interested in her job than my needs</td>
<td>2.100</td>
<td>M</td>
</tr>
<tr>
<td>20 I felt the nurse could tell when something was bothering me</td>
<td>3.900</td>
<td>H</td>
</tr>
<tr>
<td>21 I could tell this nurse wanted to make me comfortable</td>
<td>3.870</td>
<td>H</td>
</tr>
<tr>
<td>22 I felt secure and reassured with the nurse taking care of me</td>
<td>3.870</td>
<td>H</td>
</tr>
<tr>
<td>23 I felt the nurse really valued me</td>
<td>3.800</td>
<td>H</td>
</tr>
<tr>
<td>24 I felt frustrated by the nurse’s attitude*</td>
<td>1.630</td>
<td>H</td>
</tr>
<tr>
<td>25 I have not heard any offensive words or comments from the nurse or the doctor</td>
<td>3.170</td>
<td>H</td>
</tr>
</tbody>
</table>

* Item reversed measuring scale (i.e. Negative Response), and that revere an assessments scores.
countries that have revealed that continuity of midwifery care, continuous support during labor, a good support during labor and birth are more likely to lead to less pain to women and reported higher perception of control and be more satisfied with their intra partum care\(^{(1,2)}\). In addition, another studies showed that emotional support, in holding the patient’s hands. Making a difference during labor, also Nurse’s interpersonal skills are more important than technical skills for mother and baby. Due to women’s perception to labor pain were different among women in a cultural, education, social and psychological dimensions, and the responses may be influenced by several factors that include, culture, information they have about labor pain, fear of labor process, and unfamiliar environment that can worsen the situation, therefore the emotional and physical support given to labor woman by nurse or people around her is very important \(^{(3,4)}\). Further, another study reported that having received helpful labor coping measures that were valued by participants included performing roles of emotional supported, comforters, information and advice, professional technical skills, and advocates \(^{(5)}\).

**Conclusions**

The study concluded that there are a positive evaluation observed regarding women’s perception with childbirth experience after utilization pain management practices.

**Conflict of interest:** Nil

**Source of funding:** the source of funding is self

**Ethical clearance:** is obtained from the Ministry of Health / Al-Russafa Health Directorate (Al-Elwyia maternity teaching hospital), and All laboring women participants in the research - have been approved before the questionnaire is started.

**Recommendations**

The study recommended developing structured training program and clinical practice guideline of non-pharmacological pain management methods during labor and childbirth should be made available in English and Arabic which contains information about the proper use of non-pharmacological pain management methods and its advantages to midwives working in delivery room.

**References**


Mention the Treatment of Anger in Hospitalized Patients in the CCU department of Social Security Hospitals of Golestan Province

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Abstract

Background: Anger is one of the worst negative predictions in cardiac patients. Therefore, this study aimed to cure the anger of patients admitted to the Cardiac Care Unit.

Method: This quasi-experimental study was performed on 60 cardiac patients admitted to the heart care unit in a non-randomized sampling method in two groups (n = 30) and control (n = 30). In the test group, four to five times a day, the patient whispered to Allah Akbar or the prayers of Hazrat Zahra. In the control group, routine care was performed. The instrument for collecting information was anger questionnaire which was completed by self-report method before and after the intervention by the patients. The results were analyzed by inferential statistics (T-test, T-test and Independent T-test).

Result: Paired t-test showed a significant difference in the test group before and after the intervention (P <0.01). But in the control group there was no significant difference (P = 0.92). Independent t-test before intervention in the control and experimental group did not show a significant difference (P = 0.22), but this test showed a significant difference after the intervention (P = 0.006)

Conclusion: Considering the effectiveness of the therapeutic approach to reducing the rate of anger in heart patients, this non-therapeutic method can be used to reduce stress and psychological disturbances in patients and to increase the compatibility and relaxation of patients.

Keywords: cure, anger, heart disease, CCU.

Introduction

Violence has a negative impact on one’s health¹, ². Anger and stress are one of the worst prognosis in the first two hours after myocardial infarction³, ⁴. Because it increases the blood pressure and disturbs the heart rhythm⁵. Increased norepinephrine in conditions of anger and stress increases platelets, increased blood pressure, vascular stenosis and increased ischemia and atherosclerosis in the heart⁶. In such a situation, religious beliefs become more important than ever, helping the person understands the painful events of life and cause the patient to be encouraged. Therefore, spiritual experiences in difficult and critical conditions of life are considered as an adaptation⁷. Because the remembrance of God and thinking in the Lord leads to calmness and the creation of faith and the sense of security, hope and love and hope for life⁸.

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A wide range of spiritual health\(^9, 10\). Among the religious and spiritual sources, the most used source is prayer\(^11\). Recall the meaning of prayer and the ways in which the presence of God and his relationship with him are experienced. Belief in God and purposefulness of life, along with a sense of thought and attention to the spiritual issues of life, reduce the anxiety and psychological weakness and its complications\(^12-14\). A great deal of research suggests that mentioning and worshiping on the soul and soul of an individual can be adapted to critical situations and create meaning in life\(^15\). Paying attention to the mental health of patients is important\(^16, 17\) and Religious spiritual intervention is effective in people’s health\(^7, 10\). According to religious beliefs in the mental and emotional state of heart patients, researchers began a study entitled “The Effect of Therapeutic Counseling on the Rate of Anger in Patients Admitted to the Heart.”

Material and Method

This quasi-experimental study was conducted on patients admitted to the Cardiac Care Unit of Golestan Social Security Hospital in 2016. The research area of the heart care unit is the two social hospitals of Gorgan and Gonbad. The inclusion criteria for patients who were hospitalized for heart problems in the cardiac intensive care unit were all Iranian and Muslim, and the physiological status was fixed at the time of the implementation of the research plan, and on average, every patient was hospitalized for three days in the cardiac intensive care unit. In this study, 60 subjects were divided into two groups (n=30) and control (n=30), based on Dehkordi et al., With a power of 80%, confidence interval of 0.95%, significant level of 0.05, and effect size of 0.86 using software. G * POWER was c calculated\(^18\). And were randomly divided into two groups of test and control. The instrument for collecting information is the standard questionnaire of anger control skill, and its score is based on Likert scale. Testers must specify their answers between 5 degrees (very opposite, disagreeing, unobjectionable, agreeing, strongly agreeing), giving each of the answers (1 to 5) respectively. The score range is from 8 to 40. The low score in this questionnaire reflects the lower ability to control the anger of the subject. The dynamics of this questionnaire with Cronbach’s alpha coefficient was confirmed in a pilot study with a coefficient of 0.80 (Solhi and Mohammadali, 2016). The questionnaire was also approved by ten faculty members from the University of Azad University and the University of Medical Sciences. In this study, the researcher did not train the therapist and did not teach the group to the group who had the conditions to enter the study, after approving the plan at the university and obtaining a license and presenting them to the hospital authorities and obtaining their consent. In the experimental group after the initial status of the patients, the purpose of the research and obtaining informed consent and ensuring the anonymity of the units in the experimental and control groups before intervention were analyzed by anger test questionnaire. Then the researcher in the test group gave a prayer and encouraged the patients to pray for the prayers of Hazrat Zahra, in which more than 100 were used by Allah, and the teaching of sublanguage readings was performed with the revelation of Hamed and the expression of the 100th mention of Rawlah and Allah Allah, and of Patients are required to do this at least 5 times a day. The researcher then carefully controlled the patient’s treatment with hospital personnel in an intangible way. In the course of the intervention, the researcher was asked by asking the section about the mention of the mention, especially the mention of the letters of Hazrat Zahra. The only control group was routine medical and nursing care. Then, at the moment of the patient’s discharge from the intensive care unit and the transfer to the department or complete discharge from the hospital, both post-test groups were taken. Data were then entered into the spss16 software. After determining the normality of the data, the data were analyzed by the Krollomogrov-Smirnov test at a significant level (alpha -0.05) by covariance test, paired t-test and independent t-test.

Findings

The mean age of the research units was 59.93 ± 41.9 years with a mean hospitalization in CCU for 3 days, which was not significantly different between the two groups (Table 1). Mean anger in the intervention group before and after the intervention was 30.56±4.26 and 29.26 ± 4.4 after the intervention, which showed a significant difference (P <0.01) in the control group as well as in the control group before intervention. The rate of anger was 3.93 ± 93.93 and the post-test score was 32.2 ± 32. There was no significant difference between t-test and paired t-test (P = 0.92). There was no significant difference between the level of anger in the two groups before and after the intervention (P = 0.22). Independent t test showed significant difference between the two groups after the intervention (006 / 0 = P). Also, covariance test showed a significant difference between the two groups (P = 0.007, P = 0.12, Et = 0.12). That is, 12% of the post-test variations are due to the
Table (1) Comparison of demographic characteristics of patients admitted in CCU section in the control and experimental group

<table>
<thead>
<tr>
<th>P_VALUE</th>
<th>Control</th>
<th>Test</th>
<th>Demographic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0/87 = P</td>
<td>0/56 = P</td>
<td>0/65 = P</td>
</tr>
<tr>
<td></td>
<td>22/6+82/58</td>
<td>0/5+03/3</td>
<td>43/3%(12)</td>
</tr>
<tr>
<td></td>
<td>82/11+22/59</td>
<td>0/54+3/3</td>
<td>36/7%(11)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>56/7%(18)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>63/3(19)</td>
</tr>
</tbody>
</table>

Discussion

The results of this study showed that the mentioned treatment reduces anger in patients hospitalized in the CCU. Various studies show that patients with physical injuries and injuries go to religious beliefs. Prayer in patients causes adjustment of blood pressure, adjustment of respiration, stress reduction and improvement of patients’ quality of life(19). Studies show that religious beliefs and the use of spiritual experiences reduce mortality in patients(20). Religious beliefs such as prayer and mention the protective effect against heart disease(21).

In the meantime, various studies showed the effects of spirituality on reducing anger and anxiety in the patients(22). During cardiac arrhythmia, they are more emotionally and physically relaxed, and spiritual activity such as prayer and curative therapy can control emotional stress and reduce physical illness and reduce heart rate in these patients(23). mental stress such as anger causes a negative prognosis in cardiac patients(4). That mental exercises activate the region of the brain, which causes positive emotions and beneficial effects on mental mood and symptom relief in heart patients(24).

Harvard Medical School cardiologist Herbert believes that prayer and prayer can be beneficial, such as decreasing physician visits to relieve heart pain by about 26 percent lowering blood pressure, reducing drug use in 80 percent of patients with hypertension and heart patients, and Reduces visits by healthcare organizations to patients. He showed that people, through religious and religious practices such as prayer and prayer, relaxation method is a therapeutic way to reduce stress in patients with chronic pain, heart disease, hypertension and other stress-related illnesses(23). with regard to the effectiveness of the treatment of nurses as a professional group during the period of hospitalization, patients should be accompanied with attention to the spiritual and religious needs of individuals. Provide the conditions and conditions for performing the religious and spiritual practices of the patients in the hospital(15). From the limitations of this study, the short-term hospitalization of patients in the intensive care unit as well as the lack of a heart part in social welfare hospitals in Golestan
province, which could not continue the interventions in the heart. Therefore, it is suggested that a longer study be done in this study. Also, by adding a group that is in contact with the clergy of the hospital, compare the effects of these methods.

Conclusion

Considering the effectiveness of citation therapy on decreasing the anger rate in hospitalized patients in the heart and considering the culture and persuasion of the Iranian people with religious and religious issues, this therapy can be used to improve and reduce stress, anger and mental disorders of patients. Therefore, it is imperative that nurses, as a professional care team, discover spiritual needs of the patients and use spiritual care, such as prayer and mention, to reduce anger and aggression in heart patients.

Conflict of Interest: There is no conflict of interest between authors.

Acknowledgement: This article is part of the research project of the Student Research Committee of the Faculty of Nursing, Islamic Azad University, Ali Abad Katoul, which was conducted by the Ethics Committee of Islamic Azad University, Chalus Branch, IR.IAU.CHALUS.REC.1395.13. In the end, the researchers would like to thank and thank all the patients, colleagues at the Social Security Hospital of Gonbad-e-Kavoos and Gorgan in Golestan Province, who helped us with this study.

Source of Funding: This study was approved by Islamic Azad University. We thank the Deputy of Research and Technology of the University for Financial Support.

Ethical Clearance: IR.IAU.CHALUS.REC.1395.13

References


The Comparison of Social Support Against the Life Quality of The Spinal Cord Injury Under Stress

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²Lecturer, Department of Nursing, Khoy Branch, Islamic Azad University, Khoy, Iran, ³lecturer, Young Researchers and Elite Club, Aliabad Katoul branch, Islamic Azad University, Aliabad Katoul, Iran

Abstract

Introduction: The stress of war has caused severe problems for the individual and family. Spouses of veterans are gradually involved in family erosion. Lack of social protection and social dysfunction decreased life satisfaction and reduce intimacy between partners.

Purpose: this study has been done in the purpose of comparing social support against the veteran’s life who are under stress.

Method: This cross-sectional study was done on 79 posttraumatic stress disorder veteran wives and it was also done on 79 ordinary wives through convenience sampling. The instrument to collect the required information was the quality of life questionnaire which contained 36 SF questions and Medical Outcomes Study Social Support Survey (MOS-SS).and the needed information was collected by self-reported method.

Results: Veteran wives social support with the mean and the standard deviation was 19.3±5.1 while it was 31.1±3.7 versus the ordinary wives and this difference was significant (P less than 0.01). It showed the veteran wives quality of life with the mean and standard deviation of 46.3±6.088 while it showed 66.9±8.5the mean and standard deviation for the ordinary wives and it showed the significant difference (P less than 0.01).

Conclusions: the obtained result showed that the quality of life related to the health and social support in veteran wives is lower in compare with the ordinary wives with the same situation, therefore educational, treatment and supporting and promoting plans for this group is necessary.

Key word: social support; life quality; veterans; PTSD

Introduction

It is important to pay attention to mental health (1,2). These effects include spinal cord injury (3). Chronic diseases threaten the physical and mental health of the patient (4). Posttraumatic stress disorder is a serious reaction and perpetual response which appears because of being exposed to serious stresses (5). Posttraumatic stress disorder was identified and recognized during the civil war in the United States (6). The prevalence of this disorder in the public population is 4-8 percent (7). But this percentage is between 9 to 25 percent among the war injured ones during the first two years (8). The prevalence of that among the Vietnamese war soldiers has been reported as much as 30.1% to 14.7% (9). It has also been reported 17.1% to 15.6% for the solders coming back from Iraq war (10,11) According to the present documents more of the Iranian veterans have posttraumatic stress disorder and this disorder which is always with the veterans has a very bad effect on they themselves, their lives and their family lives (12, 13) Living with a person who suffers PTSD can transfer some of the symptoms to their wives (14). So one of the war victims is the veteran wives who are exposed to the PTSD so the veteran wives encounter lots of problems in their lives and theses...
problems become more and more by passing the time and cause them to have mental disorder\textsuperscript{15, 16} and this continual disorder can bring trauma to the wives and can cause anxiety, depression, isolation in the veteran wives\textsuperscript{17, 18}. The veteran wives will have family erosion little by little and because of the lack of social support they lose their satisfaction with the family members especially with their husbands\textsuperscript{19}. One of the anxiety of the family suffering from PTSD is mental relationship, matrimony compatibility and sexuality satisfaction which can affect their life quality\textsuperscript{12}. Most PTSD veteran wives sometimes face challenges that force them to be compatible to life because of their children and loving their husbands and some wives who cannot tolerate this hard situation decide to divorce\textsuperscript{20}. Studies have shown that most veteran wives have lack of social communications and they cannot express themselves well and they hesitate whether to continue that life or separate their husbands and in most cases the presence of their children cause them not to do so and this causes them to accept the problems and become compatible\textsuperscript{21}. An eight-year war with Iraq and its follow-ups have made many Iranian warfare died and veterans suffer from war-related Mental disorders\textsuperscript{22}. In addition to medical and Mental disorders, this disorder decreases the sufferers’ quality of life\textsuperscript{16}. So, this study that has been based on the purpose of comparing the social support and life quality of PTSD veteran wives has been done.

Material and Method

This cross-sectional study was done on two wife groups; the first group were the veteran wives and this group contained 158 PTSD veteran wives and the second group consists of 79 ordinary wives. This study was done in the year 2016. The study setting was Aliabad city, the population of the city is 150,000 people and according to the announcement of The Foundation of Martyrs and Veteran Affair, there are 96 PTSD veterans there. The method of sampling was done by census method among the ones who had inclusion criteria which was certified by a psychologist, and those samples were the veterans with documents that proved they are veterans, and not addicted to any drug and abuse substances were chosen. All participants provided informed consent after the study purpose was explained, and assurances were made of anonymity and privacy. At most, 79 people were qualified for the study. The control group was elected among the veteran wife relatives and their close friends and they had no PTSD disorder and no chronic mental disorder rand they were not addicted to any drugs.

This study information was collected by Demographic questionnaire, Medical Outcome Study Social and supportive Survey (MOS-SS).

Demographic questionnaire: This questionnaire contains information such as age, education, number of children, the length of time living with his wife, drug using, and the chronic mental illnesses.

Short form of quality of life (QOL-SF36): This questionnaire which contains 36 questions measures and evaluates the physical, mental and eight domains of life. The physical life quality consists of physical function, limitation in having a role because of the physical lack in efficiency, physical pain and general health.; and domain of psychological, life quality, contains limitation in role because of having emotional problems, social function, happiness and vividness and healthy mental. The method of giving score to them was between zero for the worst to 100 according to the questionnaire instruction SF36, and the score obtained by the samples is between 0 and 100 and the reliability of this study has strongly been confirmed and the reliability of the native form of this questionnaire by Shahgholian\textsuperscript{2014} in all aspects has firmly been confirmed and is 0.95 – 0.91\textsuperscript{23}.

Medical Outcomes Study Social Supports Survey (MOS-SS): This questionnaire was made by Sherbourne and Stewart. These supportive social tools have 19 questions and 5 sub scales. These subscales are tangible support (4 questions), which measures the financial and behavioral, exciting support (4 questions) which evaluates the positive emotion, sympathy, encouragement to express the feelings. Informing (4 questions) which evaluates guidance, informing, kindness (3 questions) which evaluate the amount of love, and positive social compatibility (4 questions) which evaluates the people who do the amusement and fun activities. This scale is a self-report tool which is used to measure the amount of agreement or disagreement of someone to any of them in five-point Likert scale (never = 1 point, seldom= 2 points, sometimes = 3 points, often = 4 points, always = 5 points). The lowest score in this is 19 and the highest score is 95. The high point in this test shows that the test is supported by the society a lot\textsuperscript{24} The reliability of sub domain of this test was reported by Cronbach’s alpha 0.91\textsuperscript{25}. All statistical procedures were performed with SPSS 16 statistical software. Using descriptive statistics independent t-test.
Findings

The mean age of the PTSD veteran wives were 39 individuals (49%), high school and guidance school education was 24 individuals (31%) primary school education 13 individuals (6%) and three of them had university degree, among the ordinary people wife, 48 (61%) had high school and guidance school education, (31%), 5 individuals were uneducated, 5 individuals (6%) had diploma and two of them (3%) had university degree.

The amount of life quality of the veteran wives with means and standard deviation was 46.3±0.88 (P<0.01). There also was seen a significant difference between the veteran wives versus the ordinary people (Table 1).

The amount of veteran wife’s social support by mean and standard deviation was 19.3 ± 5.1 and in the ordinary people it was 31.1 ± 3.7 (P<0.1).

Table (1): comparing the PTSD veteran wife’s life quality

<table>
<thead>
<tr>
<th>Dimension of the group</th>
<th>Life quality</th>
<th>P_value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Veteran wives</td>
<td>Ordinary people wives</td>
</tr>
<tr>
<td>Physical dimension</td>
<td>24.1 ± 4.9</td>
<td>31.4 ± 4</td>
</tr>
<tr>
<td>Mental dimension</td>
<td>22.2 ± 5.5</td>
<td>35.5 ± 4</td>
</tr>
<tr>
<td>Life quality general score</td>
<td>46.3 ± 8.8</td>
<td>66.9 ± 8.5</td>
</tr>
</tbody>
</table>

Discussion

Spinal cord injury affects the mental health of patients, with a prevalence of mild depression of 22.6% in the Hatefi et al(26). The result of the study shows that the PTSD veteran wives have a lower quality of life. In both the physical and mental dimensions, the veteran wives had lower quality of life in compare with the ordinary people wives. Studies have shown that the veteran wives have more excitement and it has caused them to have a lower quality of life. The results of the present study were same as Bares (2011), Brow (2010) and in all these studies the PTSD veteran wife’s life quality was low(9, 27).

Clinical experiences have shown that the veteran family, particularly their wives, suffer mental problems such as seclusion feeling, loneliness, inability, sin feeling, and depression(28), because the PTSD veteran wives are exposed to stresses and have lots of turmoil in their marriage lives(15). Lots of studies show that the PTSD veteran wives suffer a lot from mental disorder and also as the physical problems and it is known as the prevalent headache and physical complaint among the veteran wives(27) because living for a long time with someone who suffers post-traumatic stress disorder has a deep and serious influence on their family and can be a chronic stress factor for their wives and can be the source of big and serious problems their families(31).

This study showed that the social support for the veteran wives is lower than ordinary people wives. Communication disorder, physical problems and its consequence, lack of understanding the veterans by some people in the society is one of the most important things that the veterans suffer from(15). Social supports may have a very important effect on developing care health and also on mental pathology in reaction to the stressful life events(32). Social supports from the authorities and the social organizations can increase respect, can reduce blame and label and it can also form a better self-compatibility in veteran wives and it also can reduce stress in their lives(16).

Conclusion

According to the findings of this study, it can
be concluded that the martyr and veteran families are special families with special stresses and the amount of their stress increases day by day. Women are the center of these stresses, and they themselves and their children are at the risk of danger therefore the society is at danger. Therefore, in order to reduce the amount of stress and the factors that causes stress and anxiety in this group, presenting more and more suitable services and economical is an evitable necessity. Besides, in order to reduce the damage of stresses, teaching educational services, that is teaching life skills are among the communicative skills and stress management and doing actions on beliefs and their attitude towards the incidents and life meaning will be useful. It is also suggested that the veteran wife’s life compatibility is to be investigated through a quantitative research.

**Conflict of Interest:** There is no conflict of interest between authors.

**Acknowledgment:** This article is the result of Research Project No. 94413 Approved by the Young Researchers Club, Islamic Azad University of Aliabad Katoul. We appreciate the cooperation of the Foundation of Martyrs and Veterans Affairs and the participants in conducting this research.

**Source of Funding:** This work was supported by the Young Researchers Club, Islamic Azad University of Aliabad Katoul.

**Ethical Clearance:** Ethical considerations in this study included obtaining written consent from participants, assuring them of the confidentiality of their data, and allowing them to freely participate in this research.

**Reference**

15. Klarić M, Frančišković T, Černi Obrdalj E, Petrić D, Britvić D, Zovko NJPD. Psychiatric and health impact of primary and secondary traumatization in wives of veterans with posttraumatic stress


Risk Factors Related To Mastalgia

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Professor, Hammurabi Medical College, University of Babylon

Abstract

Background: Breast pain becomes more complicated in many humans. Various hospitals are planning for surgery in the initial stages. The main aim of this paper to find the various factors which cause mastalgia, this is mainly causing the abnormal conditions of the breast.

Method: The experiments conducted on 400 patients and this is collected from various surveys, for patients older than the age of 40 the mammography and ultrasonography (US).

Results: The mean age was 44.23 ± 9.23 years. This group consists of 300 cases, the asymptomatic group consists of 100 persons. Due to the various reasons that are related to mastalgia (p < 0.05). These are based on their breastfeeding to the child. If it is more than 4 times or more were higher in the mastalgia group (p < 0.04). The breast imaging-reporting and data system (BI-RADS 2) mammography results were associated with mastalgia (p < 0.05). These two are more common in the mastalgia group (p < 0.05) that is Fibro cysts and fibro adenomas. Based on the previous history the malignant breast disease was simultaneously higher in the mastalgia group (p < 0.05).

Conclusions: Life stress, high caffeine consumption, smoking, lactation frequency, and benign disorders were factors identified to be related with mastalgia. Though a significant relation between mastalgia and malignant breast disease was detected in our study, more studies are still necessary to investigate this relationship.

Keywords: Mastalgia · Surveys · Mammography. US findings. Pain · Surgery

Introduction

The most common factor in women is Breast pain nothing but mastalgia which causes breast tenderness, hard burning sensation or tightness in the tissue, the pain may increase or maybe constant and this will occur some other times.1 Due to the pain in the breast and tenderness, many women are fear with early symptoms of breast cancer, generally, these are not considered as disorders.2-3

Mastalgia is classified into two types such as cyclic breast pain (CBP) or non-cyclic breast pain (NCBP). CBC affects women at the age of 20 to 30 and it is up to 40 also. But still, it is menstruating. Mainly the pain occurs at the end of the menstrual cycle week or before the period occurs. At this time the breast is affected with the painful, tender, and swollen, based on the cycle the symptoms improve at another point. The pain occurs at the breast outer and upper parts of two breasts and this involve the underarm area as well. In the luteal period, the result of water increased in breast stroma produced by increasing hormone levels.3-5 Menopause is the non-cyclic mastalgia. This may like sharpness, burning, or soreness in the breast.

Causative pathophysiology of pain stays hazy however is believed to be known with secretion factors (e.g., hormone-substitution treatment, oral contraceptives, pregnancy, menses, pubescence, and menopause) these variables will modify the steroid, Lipo-Lutin and prolactin levels.6 Kind breast issue (e.g., fibrocystic changes), mind-expanding drugs, psychosocial factors and passionate stress square measure connected with breast torment.2-7 Lactation problems (e.g., engorgement, mastitis, and breast ulcer), inflammation (Tietze disorder), Postthoracotomy disorder, Spinal and paraspinal issue and Referred torment (e.g., pneumatic, heart, or vesica sickness) will likewise cause breast torment. Caffeine and vasoconstrictive utilization square measure likewise
viewed as related with pain. Cancer is a rare reason for breast torment. Breast pain associated with malignant growth is one-sided, non-cyclic, and extremely a lot of confined.

The fundamental center is to research the components of inpatient history that influences the event of breast pain, and to decide the connection among mastalgia and considerate or malignant breast utilizing a huge arrangement of members.

Method

The research was completed in an exceedingly non-public Surgery facility in Iraq, between September 2018 and August 2019. This experiment was affirmed by the local human morals committee. The patients were relegated to at least one of 2 gatherings: the pain gathering, from women, admitted to the breast surgery outpatient center with mastalgia (n = 300); and therefore the symptomless gathering (the management gathering), including patients undergoing routine breast screening (n = 100). Patients UN agency was treated with oral contraceptives, psychoactive specialists, secretion medical care, or vas operators, even as patients below 18, or over 70 older were banished from this research.

A standard summary study for all patients within the groups was performed regarding their enthusiastic anxiety, utilization of caffein and phytotoxin. AN intensive physical assessment was performed for all patients. The gap between the areola and therefore the os indent was calculable, any length surpassed 33 cm was thought of as macromastia. Breast screening was done by means that of the diagnostic procedures for patients skilled than the age of 40; imaging (US) was done once necessary for any age. Breast thickness bit by bit will increase to type D, during this manner, drop-off the affectability of the diagnostic procedure. What is more, the imaging discoveries in the diagnostic procedure were ordered accordant to the Breast Imaging-Reporting and information system (BI-RADS)? The BI-RADS classes are characterized as BI-RADS zero, fragmented; BI-RADS one, typical; BI-RADS2, thoughtful finding(s); BI-RADS three, presumptively generous; BI-RADS four, suspicious abnormality; BI-RADS five, deeply resembling harm; and BI-RADS vi, presence of biopsy-demonstrated danger.

The NCSS 2007, and PASS 2008 Statistical Software (Utah, USA) programs were used for measurable examination to perform the mean, variance, return and proportion, the Mann-Whitney U take a look at was used to affirm a normal dispersion condition. Fisher’s actual take a look at, Pearson’s chi-squared take a look at, and Yates’ progression rectification were used for the subjective evaluations of the data. Contrasts were thought of statistically vital once p < 0.05.

Results

Overall 400 females with a males period of 44.23±9.23years (approx 19-65) were enlisted. The mastalgia gathering, 300 patients, and, the well gathering, two hundred patients. Guys age was basically higher within the pain gathering (p = zero.001) (Table 1). The rate range of patients World Health Organization admitted extended gung ho feeling of tension was higher within the pain gathering (p < zero.005). alkaloid use and smoking were known with pain (p < zero.05). The lactation repeat rate disclosed associate degree on a awfully basic level higher association with bosom torment within the pain gathering (p < zero.05). The sort, shape, and laterality of bosom torment within the pain bundle area unit consolidated in Table a pair of. the speed range of patients of the pain bunch eaten soul, paracetamol, and non-steroid assuaging medicine were primarily higher (p < zero.05) (Table 3).

A total of 280 patients was given to mammographic screening, 209 patients from the pain gathering, and seventy one patients within the well gathering. The unendingly visit bosom thickness configuration was composed associate degree within the well gathering, and sort B saw as increasingly typical within the pain gathering (p < zero.01). In step with the BI-RADS categories, BI-RADS one was increasingly customary within the well occasion and BI-RADS a pair of within the commanding within the pain gathering (p < zero.05). The analysis of the U.S.A. photos disclosed the events of elementary bruises and fibro adenomas were higher within the pain gathering (p < zero.05). The assessment of the tomography discoveries between the 2 social occasions shows up in Table four.

Different sorts of six patients resolved to own BI-RADS four were within the pain gathering. Once the diagnostic assay, the psychoneurotic evaluations disclosed that 3 of those patients had a unsafe contamination, agitating cautious intervention.
Table 1. The comparison between the mastalgia and asymptomatic groups patients

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Mastalgia</th>
<th>Asymptomatic</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age mean ± SD, years</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>44.23±9.23</td>
<td>45.25±9.50</td>
<td>42.85±10.20</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Patients, n</strong></td>
<td>400</td>
<td>300</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td><strong>Stress, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>none</td>
<td>85 (20.5)</td>
<td>52 (17.3)</td>
<td>33 (33.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>stressful</td>
<td>315 (79.5)</td>
<td>248 (82.7)</td>
<td>67 (67.0)</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Caffeine consumption (coffee), n (%)</strong></td>
<td>250 (62.5)</td>
<td>170 (56.7)</td>
<td>80 (80.0)</td>
<td>0.003</td>
</tr>
<tr>
<td>none</td>
<td>65 (16.2)</td>
<td>60 (20.0)</td>
<td>5 (5.0)</td>
<td>0.012</td>
</tr>
<tr>
<td>≥ 2 cups/day</td>
<td>85 (21.3)</td>
<td>70 (23.3)</td>
<td>15 (15.0)</td>
<td>0.012</td>
</tr>
<tr>
<td><strong>Smoking (cigarettes), n (%)</strong></td>
<td>None</td>
<td>280 (70.0)</td>
<td>205 (68.3)</td>
<td>75 (75.0)</td>
</tr>
<tr>
<td>5-10/day</td>
<td>71 (17.8)</td>
<td>55 (18.3)</td>
<td>16 (16.0)</td>
<td>0.125</td>
</tr>
<tr>
<td>&gt;10/day</td>
<td>49 (12.2)</td>
<td>40 (13.3)</td>
<td>9 (9.0)</td>
<td>0.007</td>
</tr>
<tr>
<td><strong>Lactation (breast-fed infants), n (%)</strong></td>
<td>0</td>
<td>30 (7.50)</td>
<td>10 (3.30)</td>
<td>20 (20.0)</td>
</tr>
<tr>
<td>1</td>
<td>39 (9.80)</td>
<td>22 (7.30)</td>
<td>17 (17.0)</td>
<td>0.005</td>
</tr>
<tr>
<td>2</td>
<td>97 (24.2)</td>
<td>65 (21.6)</td>
<td>32 (32.0)</td>
<td>0.154</td>
</tr>
<tr>
<td>3</td>
<td>72 (18.0)</td>
<td>60 (20.0)</td>
<td>12 (12.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>≥ 4</td>
<td>162 (40.5)</td>
<td>143 (47.7)</td>
<td>19 (19.0)</td>
<td>0.035</td>
</tr>
<tr>
<td><strong>BSO±TAH, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>384 (96.3)</td>
<td>294 (98.0)</td>
<td>90 (90.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Yes</td>
<td>16 (4.0)</td>
<td>6 (2.0)</td>
<td>10 (10.0)</td>
<td>0.005</td>
</tr>
<tr>
<td><strong>Menopause, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>premenopausal</td>
<td>312 (78.0)</td>
<td>252 (84.0)</td>
<td>60 (60.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>postmenopausal</td>
<td>88 (22.0)</td>
<td>48 (16.0)</td>
<td>40 (40.0)</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Breast size, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>368 (92.0)</td>
<td>280 (93.3)</td>
<td>88 (88.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>macromastia</td>
<td>32 (8.0)</td>
<td>20 (6.7)</td>
<td>12 (12.0)</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Recent breast trauma, n (%)</strong></td>
<td>No</td>
<td>391 (97.8)</td>
<td>292 (97.3)</td>
<td>99 (99.0)</td>
</tr>
<tr>
<td>Yes</td>
<td>9 (2.2)</td>
<td>8 (2.7)</td>
<td>1 (1.0)</td>
<td>0.058</td>
</tr>
<tr>
<td><strong>Breast cancer, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>393 (98.2)</td>
<td>295 (98.3)</td>
<td>98 (98.0)</td>
<td>0.015</td>
</tr>
<tr>
<td>Yes</td>
<td>7 (1.8)</td>
<td>5 (1.7)</td>
<td>2 (2.0)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

SD, BSO±TAH: history of a prior bilateral salpingooophorectomy with or without total abdominal hysterectomy.
Table 2. Mastalgia patients’ based on the pain the distribution is done

<table>
<thead>
<tr>
<th>Breast Pain</th>
<th>Type</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiation</td>
<td>Diffused</td>
<td>140</td>
<td>46.7</td>
</tr>
<tr>
<td></td>
<td>Localized</td>
<td>160</td>
<td>53.3</td>
</tr>
<tr>
<td>Laterality</td>
<td>Bilateral</td>
<td>61</td>
<td>20.3</td>
</tr>
<tr>
<td></td>
<td>Unilateral</td>
<td>239</td>
<td>79.7</td>
</tr>
<tr>
<td>Relation with menstruation</td>
<td>Cyclic</td>
<td>205</td>
<td>68.3</td>
</tr>
<tr>
<td></td>
<td>Non-cyclic</td>
<td>95</td>
<td>31.7</td>
</tr>
</tbody>
</table>

Table 3. Analgesic consumption comparison between the mastalgia and asymptomatic groups

<table>
<thead>
<tr>
<th>Analgesic consumption n (%)</th>
<th>Total</th>
<th>Mastalgia</th>
<th>Asymptomatic</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>350 (87.5)</td>
<td>255(85.0)</td>
<td>95(95.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Aspirin</td>
<td>9(2.2)</td>
<td>8(2.7)</td>
<td>1(1.0)</td>
<td>0.011</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>17(4.3)</td>
<td>15(5.0)</td>
<td>2(2.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>NSAIDs</td>
<td>24(6.0)</td>
<td>22(7.3)</td>
<td>2(2.0)</td>
<td>0.021</td>
</tr>
</tbody>
</table>

Table 4. Comparison of mammography and ultrasound findings between the mastalgia and asymptomatic groups

<table>
<thead>
<tr>
<th>Mammographic breast density (pattern) (n=280)</th>
<th>Total, n (%)</th>
<th>Mastalgia, n (%)</th>
<th>Asymptomatic, n (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>57 (20.4)</td>
<td>12 (5.7)</td>
<td>45 (63.4)</td>
<td>0.001</td>
</tr>
<tr>
<td>B</td>
<td>172 (61.4)</td>
<td>160 (76.6)</td>
<td>12 (16.9)</td>
<td>0.001</td>
</tr>
<tr>
<td>C</td>
<td>44 (14.7)</td>
<td>34 (16.3)</td>
<td>10 (14.1)</td>
<td>0.010</td>
</tr>
<tr>
<td>D</td>
<td>7 (2.5)</td>
<td>3 (1.4)</td>
<td>4 (5.6)</td>
<td>0.020</td>
</tr>
<tr>
<td>Mammographic category (n=280)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BI-RADS 0</td>
<td>160 (57.1)</td>
<td>101 (52.3)</td>
<td>59 (67.8)</td>
<td>0.073</td>
</tr>
<tr>
<td>BI-RADS 1</td>
<td>30(10.7)</td>
<td>16 (8.30)</td>
<td>14 (16.1)</td>
<td>0.001</td>
</tr>
<tr>
<td>BI-RADS 2</td>
<td>70(25.0)</td>
<td>60 (31.1)</td>
<td>10 (11.5)</td>
<td>0.001</td>
</tr>
<tr>
<td>BI-RADS 3</td>
<td>10(3.57)</td>
<td>7 (3.63)</td>
<td>3 (3.45)</td>
<td>0.014</td>
</tr>
<tr>
<td>BI-RADS 4</td>
<td>6(2.14)</td>
<td>6 (3.11)</td>
<td>0 (0.0)</td>
<td>0.021</td>
</tr>
<tr>
<td>BI-RADS 5</td>
<td>4(1.4)</td>
<td>3 (1.55)</td>
<td>1 (1.15)</td>
<td>0.002</td>
</tr>
<tr>
<td>Ultrasound findings (n=285)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>35(12.3)</td>
<td>25 (13.5)</td>
<td>10 (10.0)</td>
<td>0.003</td>
</tr>
<tr>
<td>Simple cysts</td>
<td>20(7.02)</td>
<td>20 (10.8)</td>
<td>0 (0.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Fibroadenomas</td>
<td>45(15.8)</td>
<td>35 (18.9)</td>
<td>10 (10.0)</td>
<td>0.039</td>
</tr>
<tr>
<td>Fibrocystic disease</td>
<td>180(63.2)</td>
<td>100 (54.1)</td>
<td>80 (80.0)</td>
<td>0.015</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>3(0.78)</td>
<td>3 (1.62)</td>
<td>0 (0.0)</td>
<td>0.014</td>
</tr>
<tr>
<td>Complex cysts</td>
<td>2(0.70)</td>
<td>2 (1.08)</td>
<td>0 (0.0)</td>
<td>0.004</td>
</tr>
<tr>
<td>Fatty tissue necrosis</td>
<td>1(0.35)</td>
<td>1(0.54)</td>
<td>0 (0.0)</td>
<td>0.004</td>
</tr>
</tbody>
</table>
Discussion

Various factors are effecting mastalgia, The author portrayed that the period of women grumbling of the pain in breast was in the range 35-55 years and that this side effect was once in a while experienced younger than 45. Present research, the male age of the all-out investigation group was 44.23±9.23years (territory 19-65 years), and the male age of the mastalgia group was higher lighting up that mastalgia happens in the perimenopausal period when the inconsistence of hormonal and menstrual beginnings. Numerous studies showed that there is a connection between breast torment and mental strain. In this paper, the patients how had an unpleasant lifestyle were essentially higher in the mastalgia gathering. Numerous concentrates uncovered a connection between caffeine utilization and smoking with breast torment. In our examination, there was a critical association between either standard caffeine utilization or substantial smoking (in excess of 10 cigarettes every day) and mastalgia.

Anomalies of the estrogen/progesterone proportion, which can happen on the off chance that one of these hormones increment, the other one will diminish, or expanded prolactin levels are identified with mastalgia. The event of breast side effects, for example, expanding, irritation, and nodularity in the menopausal period, at that point the end of these manifestations in the postmenopausal period is potential because of the impacts of the estrogen hormone.

In perspective on lactation, the level of ladies with a background marked by breast feeding or more newborn children were expanded in the mastalgia gathering. These outcomes shed light on the connection of breast torment with the expanded frequencies of lactation, which is identified with an expanded number of births, and therefore with an expanded introduction to significant levels of estrogen and prolactin. Additionally, at least 4 times of lactation may bring about anatomical changes in the breast tissue, particularly in the ductal framework, which could likewise expand the breast torment episodes.

An earlier reciprocal salpingo-oophorectomy with or without absolute stomach hysterectomy was identified with a fundamentally diminished event of mastalgia.

The mastalgia side effects which coordinate the announced investigations that found a huge connection between the asymptomatic gathering and postmenopausal state.

Macromastia or breast hypertrophy is an uncommon ailment of the breast connective tissues wherein the breasts become exorbitantly enormous. In the event that the separation between the sternal score and the areola surpassed 33 cm this means that macromastia. In our examination, the recurrence of macromastia was altogether lesser in the mastalgia gathering, recommending that macromastia probably won’t be one of the components that reason mastalgia. Mastalgia may happen attributable to greasy tissue rot, or a strain in the Cooper tendons as aftereffects of either dull or entering injury to the breast. In our investigation, higher paces of injury history found in the mastalgia gathering.

Extreme mastalgia can happen in 10–22% of patients and requires medicinal medications. As an initial step approach, analgesics In our present examination, the mastalgia bunch utilized pain-relieving specialists more than the asymptomatic gathering, and the most picked pain relieving type was NSAIDs.

As breast thickness builds, the mastalgia does. In our examination, the sort B breast design was higher in the mastalgia gathering. Different designs demonstrated immaterial contrasts between the two gatherings. Mastalgia and generous breast issue is altogether related. Our examination indicated a higher recurrence of mastalgia in patients with discoveries of benevolent issue (BI-RADS 2). The connection between breast torment and kind issue distinguished utilizing the US is likewise easily proven wrong .The author announced that fibroadenomas and basic sores were the most widely recognized existing together benevolent sores.

Furthermore, the rate of fibrocystic malady recognized by the US was essentially higher in patients with mastalgia. Since the fibrocystic ailment is a substance demonstrating a summed up inclusion of the breast parenchyma, that it prompts diffuse breast torment isn’t startling. Despite the high likelihood of the dwelling together of a dangerous breast tumor with non-cyclic breast torment, albeit uncommon, repetitive mastalgia can likewise be identified with harm.

Despite the fact that the aftereffects of our examination uncover a relationship among mastalgia and danger, this issue is as yet dubious, and there is as yet a requirement for enormous future investigations to uncover the relationship more precisely.
Conclusion

Symptoms like stress, coffee consumption, smoking, and other abnormal disorders are the key points found which is related to mastalgia. There is a relation between mastalgia and malignant breast disease is found in our research. In the future, more research is to be done.

Acknowledgement: Disclosure Statement

All writers announce that no issues are found in that and they have no direct or business monetary affectation related to distributing this article.

Conflict of Interest: The author would like to state that there was no conflict of interest whatsoever.

Ethical Clearance: The author has obtained ethical clearance from the committee

Source of Funding: The author has used her own funds to do this research.

References

Genetics Risk Factors and Progression of Renal Failure

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Abstract

Objective: “MYH9 gene” is “expressed” in “kidney, platelets and liver and in lesser amounts in the thymus, spleen, and intestine”, its responsible for encoding a protein called “non muscle myosin heavy chain”. To evaluate the role of MYH9 SNP on developing of renal failure.

Method: This study depending on “methodology of Case-control study”, subjects involved were one hundred as patients and control; 50 “patients” complaining renal failure and 50 apparently healthy controls. DNA was extracted from venous blood. The “MYH9 gene polymorphisms” were recognized by applying the procedure of (“PCR-RFLP”).

Result: Genotype at rs4821480 in patients with RF: finding that obtained were TT (59%), GT (34%), and GG (6.0%) and for control TT (45%) GT (40%), GG(15%). This analysis of data indicated the TT genotype homozygote at rs4821480 convenes independently a threatening of RF than does the GT and GG genotypes. a variation in the genotype at rs3752462 was shown in patients with RF: CC (4.8%), CT (73.2%), and TT (22.0%).

Conclusion: The outcomes indicate that the CT genotype at rs3752462 confers independently a risk factor of RF than those of TT and CC genotypes. There is no significant correlation between distribution of alleles and age, sex, resident, jobs, smoking habit, family history, body mass index (BMI), and medical history (P>0.05).

Keywords: MYH9 SNP, renal failure, allele distribution and genotyping.

Introduction

The elucidation of chronic kidney disease in the last period had been simplified for assessing its proof of identity and final explanation of chronic renal failure as it is correlated with drop of glomerular filtration rate over 3 months. in adult the diagnosis of renal failure confirm when filtration of kidney drop to “less than 60 mL/min/1.73 m²” and for rate less than 60 mL/min/1.73 m² can considered renal failure with some other signs that show renal defect as abnormal of x-ray or urine sediment or biopsy of renal alongside other finding 1.

For the progression of different renal diseases with aid of final attitude of recent researches that intact “actin cytoskeleton” is a crucial to maintain the typical purpose of podocyte building and filtration 2. The ‘nonmuscle myosin heavy chain 9 (MYH9) gene” translate “nonmuscle myosin protein”, which expressed in body cells and binds to “actin cytoskeleton” to achieve specific “intracellular motor functions” 3.

Former workings were indicted a number of kidney diseases such as “(May-Hegglin, , Fechtner and Sebastiana syndromes)” linking with MYH9 mutations, also approached to identifying the link between “podocyte injury and MYH9 mutations“, which suggested that mutation in this gene that cause fluctuations of protein then diminishing of the “glomerular filtration barrier”, from this defect “proteinuria and/ or haematuria” are developing, and even “renal failure” in advanced deterioration of kidney function 4, 5. And the effort of newly works of a “genome-wide association study (GWAS)” was recognized “MYH9” as a foremost predisposition gene for ESRD, in different kinds of nephropathy as ‘idiopathic focal segmental glomerulosclerosis, HIV-associated nephropathy and hypertension” in different ethnicities ‘(African-
Americans, Europeans and Hispanic Americans)" these works suggested a link between the glomerular function and MYS gene 6–9. "MYH9 gene", comprise from 40 "exons on “chromosome 22 q12.3-13.2”, its translate of “non-muscle myosin heavy chain protein” with approximately “224,000 kDa” molecular weight, this protein dimerizes to form a chief motor protein (motor domain of non-muscle myosin IIA), that distributed in various cells 10. This gene mainly expressed in “fibroblasts, erythroblasts, and kidney cells” 11. “cytoskeleton” impairment developed in a case of irregularity in “expression, positioning, or function” and as a result of this impairment “proteinuria, haematuria, or renal failure” developed as listed in several situations12.

Anomalous in “MYH9” expression considered major predisposing factor for developing and progression in the function of kidney according to the "genome-wide association studies (GWAS)” documentations that established on numerous nephropathies, including “idiopathic focal segmental glomerulosclerosis”, “human immunodeficiency virus” (HIV)- nephropathy. The previous relation verified in different society as “African-Americans and Hispanic Americans to Europeans” 13-16.

Table 1. Forward and reverse primers of MYH9 gene polymorphism

<table>
<thead>
<tr>
<th>Primers Sequences</th>
<th>MYH9 gene polymorphism</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>CCGCTGGGCAAGGGGTGT TCTTCTGTGAGTTGTG GGTG</td>
</tr>
<tr>
<td>R</td>
<td>CCAGGAGCATCGGGTCTTA F</td>
</tr>
<tr>
<td>F</td>
<td>CACCTCCACAACCAACAGAGCT</td>
</tr>
<tr>
<td>R</td>
<td>rs4821480</td>
</tr>
<tr>
<td></td>
<td>rs3752462</td>
</tr>
</tbody>
</table>

Results

The amplification outcome of rs3752462 with selected forward and reverse primer was 421 base pair band as figure (1) reveal.
According to the restriction digestion pattern of rs3752462 polymorphism which is revealed in figure (2 and 3), genotypes of rs3752462 were divided into 3 groups:

1- Two bands (243 and 178 bp) are homozygote (CC).
2- Three bands; (243, 92 and 86) are homozygote (TT).
3- Four bands (243, 178, 92 and 86) are heterozygote (CT).

Figure (2): Restriction digestion of PCR products demonstrating the patterns of different genotypes of MYH9 on 2% agarose, 100V; bands of 243+178 bp (CC), 243+92+86 bp (TT) and 243+178+92+86 bp (CT).

Figure (3): Restriction digestion of PCR products demonstrating the patterns of different genotypes of MYH9 on polyacrylamide gel; bands of 243+178 bp (CC), 243+92+86 bp (TT) and 243+178+92+86 bp (CT).

The product of rs4821480 PCR after incubated with restrictive endonuclease revealed fragment sizes of different alleles, figure (5) which are recognized as band of different size and accordingly the alleles are classified as follow:
Figure (4). RFLP pattern of Polymorphism (rs4821480) MYSgene genotypes. Restricted fragments were electrophoresed (lane A. ladder, lane B. GT allele, lane C. TT allele) Genotyping of MYH9 of rs4821480 are representing in table

Table (2) Comparison of Alleles and Genotype Frequency of (rs4821480) of MYH9 Polymorphism in renal disease and control groups

<table>
<thead>
<tr>
<th>Genotype</th>
<th>CKD no. (%)</th>
<th>Control no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>% 32 61.5</td>
<td>38% 16</td>
</tr>
<tr>
<td>GT</td>
<td>32.6% 17</td>
<td>52.4 22</td>
</tr>
<tr>
<td>GG</td>
<td>5.7% 3</td>
<td>9.5% 4</td>
</tr>
</tbody>
</table>

Allele distributions and Genotype of the MYH9 polymorphisms rs3752462 revealed that statistical variances between control and RF subjects were non-significant in terms of genotype or allele distribution. The most common one was CT with prevalence of 69.7% in control group and 73.2% in the RF group. the genotype of CC at rs3752462 is a lower risk of CKD than TT and CT genotypes that were suggested from current results. Findings refer to that genotype (CT) at rs3752462 is independent risk factor for CKD. This finding put forward that the CC genotype may be a defending reason against renal failure than
does the TT and CT genotypes.

There is no significant correlation between alleles distribution at rs4821480 and rs3752462 and Age, sex, resident, jobs, smoking habit, family history, body mass index (BMI), medical history (P> 0.05).

**Discussion**

In recent years from the effort of scientist work, there was increasing suggestion involved the beneficial of genetic factors on development and progression of a disease as widely considered to be a “polygenic” disorder. The conclusion about developing of many diseases related to environmental and genetic cooperated these factor lead to risk of developing the disease.

This work is directed to investigate one of genetic influencing factor associated with CKD so we select MYH9 SNP and its role on developing and progression of renal failure as these difficulties are predisposing to increasing in morbidity and mortality and reflected a health problem in society.

MYH9 gene which codifies the “myosin-IIA protein” that contain an “IQ domain” which responsible on its biological function these related to role of catalytic action of enzyme present in the podocyte foot that contribute to filament movement. In animal studies, mutations in MYH9 are related to “phenotypic kidney abnormalities including albuminuria and FSGS” 21-22, as well as defects in morphogenesis 23. The “pathogenesis” of MYH9-related kidney disease is not fully assumed. In spite of establishing the role of MYH9-related disorders transformation the “podocyte cytoskeleton” and as a result lead to “glomerular filtration barrier damage that basis for developing: proteinuria, hematuria, and finally to renal failure” 24. There was no significant variances between the healthy control group and the renal failure group in terms of “genotype or allele distributions at rs3752462 of MYH9 gene” and this similar to finding of Chinese study 25, but person who have CT genotype consider risky for development of CKD and need extensive care than other with CC or TT genotype so we can search for gene therapy and avoid the bad progression.

The association between MYH9 polymorphism and developing renal failure confirmed with other study 26-29

**Ethical clearance**

Approval by scientific committee of Babylon Medical College (University of Babylon, Iraq) and the Biochemistry Department in the medical college.

**Source of Funding**: The funding body written out in full by self

**Conflict of Interest**: If any then mention it otherwise write it as nil

**References**

8. Kopp JB, Smith MW, Nelson GW, Johnson RC,


The Diagnostic Accuracy of Phalen Test and Prayer Test in Carpal Tunnel Syndrome

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Abstract

Background of Study: Carpal Tunnel Syndrome is a series of symptoms and signs because of pressure on the median nerve in a small space in the wrist called carpal tunnel. In diagnosing CTS, it is required anamnesis and thorough physical examination which includes CTS provocation test, for instance Phalen test, Prayer test, and Tinel sign. The aim of this study is to reveal the diagnostic accuracy of Phalen test and Prayer test in diagnosing CTS.

Methods: The samples that fulfilled inclusion criteria were 19 subjects went through Phalen test, Prayer test, and nerve conduction study using CSI in Department of Physical Medicine and Rehabilitation Dr. Soetomo General Hospital Surabaya, Indonesia. The nerve conduction study using CSI was conducted by EMG Cadwell equipment. The data analysis was conducted by using SPSS software.

Result of the Study: The sensitivity of the Phalen test and Prayer test combination in diagnosing CTS was 94.1%. The specificity of Phalen test and Prayer test combination in diagnosing CTS was 100%. The positive predictive value was 100% while the negative predictive value was 66.7%. The result of person chi-square examination was obtained asymp. Sig value of 0.001 < α 0.05.

Conclusion: There was a correlation between the combination of Phalen test and Prayer test with CTS.

Keywords: Carpal Tunnel Syndrome, Combined Sensory Index, Phalen test, Prayer test,

Introduction

Carpal Tunnel Syndrome is a series of symptoms and signs because of pressure on the median nerve in a small space in the wrist called carpal tunnel. The common symptoms include numbness, paresthesia, or the pain in distributed median nerve in the hand ¹,².

CTS has been encountered by 3% of the entire population ³. CTS is considered the most frequent cases than other entrapment neuropathy⁴,⁵. There has been no specific data which specifies the number of CTS patients in Indonesia. However, there were 173 cases of CTS in Dr. Soetomo General Hospital Surabaya on October 2011 until September 2012. Only 55% of them (95 cases) visited the Medical Rehabilitation Unit. In addition, CTS is mostly encountered by females aged 40-60 years old (70%) as from 173 cases in Dr. Soetomo General Hospital Surabaya, 151 cases (87%) of the entire cases were females. CTS usually strikes both hands, however it is more severe in the dominant side of hand ⁶. The increase of carpal tunnel compression of 30 mmHg can cause venous congestion in nerve and interruptaxonal transport ⁷. Without adequate therapy, the nerve compression can cause median nerve damage with; consequently, the function of hands will be less or lost ¹.

The electro-diagnosis examination is the only method to find out the media nerve physiology caused by CTS and eliminate other diagnosis ⁷. Electrodiagnosis examination is considered as the golden standard to diagnose CTS. In fact, there are several electro-diagnosis methods in diagnosing CTS ⁸,⁹. One of the electro-diagnosis methods is CSI (Combined Sensory Index) that compare the median sensory nerve conduction to

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ulnar and radial nerve. This CSI method has the highest sensitivity and specificity than other electro-diagnosis methods. However, most hospitals in Indonesia still cannot conduct such examination method due to the unavailability of EMG machine. In diagnosing CTS, it is required anamnesis and thorough physical examination which includes CTS provocation test. The CTS provocation tests that are usually conducted in the Medical Rehabilitation Unit Dr. Soetomo General Hospital Surabaya, Indonesia are Phalen test, Prayer test, and Tinel sign. The aim of this study is to reveal the diagnostic accuracy of Phalen test and Prayer test in diagnosing CTS.

Method

This study was an observational analytic study conducted using Cross Sectional approach in Department of Physical Medicine and Rehabilitation Dr. Soetomo General Hospital Surabaya, Indonesia since November 2012 until January 2013. The sample was collected on the basis of consecutive sampling. The samples of this study were CTS patients who fulfilled inclusion criteria (patients with CTS symptoms aged 20-60 years old, no movement limitations in upper limb joins, understood and followed the test rules, and fulfilled the requirements in following nerve conduction examination) and signed the informed consent forms. Nineteen subjects who fulfilled the inclusion criteria encountered Phalen test, Prayer test, and nerve conduction study using CSI on the same day. The nerve conduction study using CSI was conducted by using EMG Cadwell equipment (xx, yy, zz). The study protocol was approved by the Ethical Commission to conduct basic science/clinical research in Dr. Soetomo General Hospital Surabaya. The analysis of data was conducted by using SPSS software (SPSS, Inc., Chicago, IL).

Result

Table 1 The characteristic of research sample

<table>
<thead>
<tr>
<th></th>
<th>Minimum</th>
<th>Maximum</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>45</td>
<td>59</td>
<td>51.7</td>
</tr>
<tr>
<td>Height(cm)</td>
<td>145</td>
<td>155</td>
<td>151</td>
</tr>
<tr>
<td>Weight(kg)</td>
<td>47</td>
<td>75</td>
<td>58.1</td>
</tr>
<tr>
<td>BMI</td>
<td>20.89</td>
<td>32.04</td>
<td>25.4</td>
</tr>
</tbody>
</table>

BMI: ......

Table 2 The result of Phalen test in CSI

<table>
<thead>
<tr>
<th>Positive</th>
<th>CSI</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phalen test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>2</td>
</tr>
</tbody>
</table>

CSI: ........
The samples were all females with the average age of 51.7, with the age range of 45–59 years old. The average BMI was 25.4, with the BMI range of 20.89-32.24.30% of samples worked as employees in particular cigarette factory in Surabaya, 20% of them worked as housemaids, 20% of them worked as housewives, and 10% of them worked as private practice midwives (Table 5.1). On the average, the subjects have encountered CTS symptoms for 19.3 months.

From 19 hands, 17 hands of them (89.5%) suffered from CTS while 2 other hands (10.5%) did not encountered CTS From 17 hands that suffered from CTS, 14 (82.4%) obtained positive Phalen test and 3 (17.6%) obtained negative Phalen test. The correct positive predictive value was 14 while the false positive value was 0. Moreover, the false negative was 3 (table 5.2).

We obtained the sensitivity value of Phalen test in CTS was 82%. The specificity value of Phalen test in CTS was 100%. The positive predictive value was 100% while the negative predictive value was 40%. The AUC value of Phalen test was 0.912 (Figure 5.1). From 17 hands that suffered from CTS, 15 (88.2%) of them were with positive Prayer test result and 2 (11.8%) of them were with negative Prayer test result. The correct positive value was 15 while the correct negative was 2. The false positive was 0 while the false negative was 2 (table 3).We obtained the sensitivity value of Prayer test in CTS was 80% while the specificity was 88%. The specificity of Prayer test in CTS was 100%. The positive predictive value was 100% while the negative predictive value was 50%. The value of AUC in Prayer test was 0.941 (Figure 5.2).

From 17 hands that suffered from CTS, 16 (94.1%) were the combination of positive Phalen test and Prayer test result and 1 (5.9%) were negative. The correct positive value was 16 and the correct negative was 2. The false positive was 0 and false negative was 1 (Table 5.4). We obtained the sensitivity value of the Phalen test and Prayer test combination in CTS 94.1 %. On the other hand, the specificity of the Phalen and Prayer test combination in CTS was 100%. The positive predictive value was 100% while the negative predictive value was 66.7%.
To examine the correlation between Phalen test and CTS was conducted through Pearson Chi Square examination. The result of person chi-square examination was obtained asymp. Sig value of 0.012 < α 0.05. It meant that there was a correlation between Phalen test and CTS.

To examine the correlation between Prayer test and CTS was conducted through Pearson Chi Square examination. The result of person chi-square examination was obtained asymp. Sig value of 0.004 < α 0.05. It meant that there was a correlation between Prayer test and CTS. To examine the correlation between Phalen and Prayer test with CTS was conducted through Pearson chi-square examination. The result of person chi-square examination was obtained asymp. Sig value of 0.001 < α 0.05. It meant that there was a correlation between the combination of Phalen test and Prayer test with CTS.

**Discussion**

All the samples were females with average age of 51.7 and with the age range between 45 – 59 years old. This demographic data was based on the result of Craig and Richardson’s study in 2011 which asserted that women are more likely to have carpal tunnel syndrome than men. They further stated that carpal tunnel syndrome is most frequently diagnosed between the ages of 40 and 60. Women are more likely to have carpal tunnel syndrome than men because the size of carpal tunnel is smaller than men’s.

The average BMI in this study was 25.4, between the ranges of BMI 20.89–32.24. Obesity is one of the factors for carpal tunnel syndrome. The risk of having CTS will increase in obesity patients. In individuals with BMI >29, the CTS risk increases 2.5 time compared to individuals with BMI <20.

All the subjects had works that required them to do repetitive motions in their wrists. 30% of the research samples worked as employees in particular cigarette factory in Surabaya. Employees of cigarette factory are much related to repetitive motions to all directions using their wrists. 20% of the samples worked as housemaids that were also related to repetitive motions. 20% of them worked as housewives and 10% worked as private practice midwives. The number of CTS risks will increase in the workers that require them repetitive and excessive hand movements.

The diagnostic test with sensitivity and specificity value of > 50 % is considered high and proved to diagnose a particular disease. The diagnostic accuracy of Phalen test and Prayer test in this study was reported in ROC (Receiver Operating Curve). The most important section in ROC is the measurement result in Area Under Curve (AUC). The vast of AUC was used to assess how accurate a diagnostic test was. A diagnostic accuracy of a test was considered high if it had the AUC value of 1.

The sensitivity value of Phalen test in CTS was 82% whereas the specificity was 100%. The value of AUC in Phalen test was 0.912 (near 1). The sensitivity in Prayer test in this study was 88% while the specificity was 100%. The AUC value of Prayer test in CTS was 0.941. It meant that Phalen test and Prayer test had high accuracy to detect the occurrence of CTS due to the value of sensitivity and specificity was each > 50% and the value of AUC was nearly 1.

The hypothesis of this study stated that Phalen test and Prayer test had high diagnostic accuracy sensitivity, and specificity in diagnosing CTS. The result of this study supported the previous study that was conducted by J. Bruske et al. about the sensitivity and specificity of Phalen and Tinel test in CTS. These samples of their study were 147 hands of 112 patients who underwent CTS operation in General and Hand Surgery, Pomeranian, Polandia from 1993 -1998. The CTS diagnosis was on the basis of electro-diagnosis with the nerve conduction study (the median sensory nerve conduction speed < 40 m/s and distal motor latency > 0.6 ms/cm). The sensitivity in Phalen test in J. Bruske et al.’s study was 85% while the specificity was 89%.

In this study, in sensitivity and specificity in Phalen test was higher than the result of J. Bruske et al. It was because, in our study, we applied electro-diagnosis examination using CSI that had higher diagnostic accuracy, sensitivity, and specificity than J. Bruske et al.’s study.

Sayeeda Bilkis, et al. in 2012 conducted a study in 66 hands of 37 patients aged more than 18 years old which found the sensitivity Phalen test of 50 %, while the specificity was 100%. Electro-diagnosis examination was considered as the golden standard. The electro-diagnosis method that was used in this study applied CSI method, in which it was the difference in peak latency between the ulnar nerve and the median nerve from the
palm of the hand to the wrist. The electro-diagnosis was considered positive if the difference in peak latency between the ulnar nerve and the median nerve from the palm of the hand to the wrist with 8 cm showing the difference of >0.3 ms\(^{15}\).

We found that the sensitivity in Phalen test was 82% which was higher than the study conducted by Sayeeda Bilkis et al.\(^{15}\). It was because we added up the comparison of the ulnar nerve and the median nerve from the palm of the hand to the forth finger, the comparison of the radialis nerve and the median nerve from the palm of the hand to the thumb, the comparison of the ulnar nerve and the median nerve from the palm of the hand to the wrist with the distance of 8 cm.

Filip Georgiew, et al.\(^{16}\) conducted a study in 215 hands from 163 CTS patients, who were confirmed by electro-diagnosis examination, stated that the sensitivity of Phalen test depends on the severity of CTS. The more severe the CTS is, it will be more sensitive. In the very mild CTS, the sensitivity in Phalen test was 63%. In the mild CTS, the sensitivity in Phalen test was 78.7%. In the medium CTS, the sensitivity in Phalen test was 97.3%, while in the heavy and very heavy CTS, the sensitivity in Phalen test was 100% (14). Because of sample limitations in our study, we did not calculate the sensitivity of Phalen and Prayer test with the CTS severity.

The result of the study stated that the sensitivity of Prayer test (88%) was higher that Phalen test (82%). It was in accordance with Denis Cerimegic et al. from Departemen Neurology of Dubrofnik, Croasia in 2010 \(^{17}\). They conducted a nerve conduction study in CTS patients in three positions: neutral position, maximum extension and maximum flexibility from the wrist. It was examined the motorist nerve conduction and sensory in median nerve in the wrist area. The electro-diagnosis result in the extension position of the wrist (Prayer position) was worse than other two positions \(^{17}\).

This study was expected in giving the benefits from scientific knowledge and enriching the information about the diagnostic accuracy, sensitivity, and specificity in Phalen and Prayer test in diagnosing CTS. In addition, it provides the contribution in the development of diagnosis management and therapy towards CTS patients. Phalen test and Prayer test had sensitivity, specificity, and diagnostic accuracy in diagnosing CTS. In the hospitals that have not provided electro-diagnosis equipments, CTS diagnosis can be conducted with thorough anamnesis, and the right combination of Phalen test and Prayer test.

**Conflict of Interest**: There is no conflict interest

**Source of Funding**: This study is self-funded

**Ethical of Clearence**: This study was approved by Ethical Commision of Health Research Faculty of Medicine University of Airlangga

**Conclusion**

1. The sensitivity of Phalen test in diagnosing CTS was 82%.
2. The specificity of Phalen test in diagnosing CTS was 100%.
3. The sensitivity of Prayer test in diagnosing CTS was 88%.
4. The specificity of Prayer test in diagnosing CTS was 100%.
5. The sensitivity of the Phalen test and Prayer test combination in diagnosing CTS was 94.1%.
6. Prayer test was more sensitive in diagnosing CTS than Phalen test was.
7. The combination of Phalen test and Prayer test in diagnosing CTS had higher sensitivity value than Phalen test itself or Prayer test itself.

**References**


Conservative & Operative Management of Tuberculous Spondylitis in Children

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Abstract

Background: Tuberculosis is a deadly disease worldwide. Tuberculosis usually occurs in the lung, but there are other tuberculosis most commonly afflicted by the patient and involves the spine and is called tuberculosis of bones and joints. Management of tuberculous spondylitis is conservative and operative.

Aim: This study aimed to compare the results of conservative and operative therapy in the management of tuberculous spondylitis in children.

Methods: The sample used was teaching with tuberculous spondylitis in Dr. Soetomo General Hospital who underwent operative and conservative therapy that became group 1 and group 2. Inclusion criteria included patients aged <18 years old, suffering from tuberculous spondylitis by conservative or operative therapy in Dr. Soetomo Hospital.

Results: In patients receiving conservative therapy, the Visual analog scale (VAS) decreased significantly (p = 0.001), while frankle improved but not significantly (p = 0.157). Cobb’s angle of patients receiving conservative therapy increased significantly (p = 0.007). Patients who received operative therapy showed significant improvement of VAS and Frankle (p = 0.001 and p = 0.011). The patient’s Cobb’s angle after surgery decreased but it was not significant (p = 0.575).

Conclusion: The role of surgical action has an important role in the treatment of tuberculous spondylitis and gives better results than conservatives in patients with pain, neurological deficits and Cobb’s angle progression.

Keywords: Cobb’s angle, neurological deficits, tuberculosis, spondylitis, VAS

Introduction

Tuberculosis is still one of the most deadly diseases in the world. According to the world health organization (WHO) in November 2010, one-third of the world’s population is estimated to be infected with tuberculosis germs. Tuberculosis manifestations are usually confined to the lung. However, it can affect any organ such as bone, genitourinary tract and central nervous system known as extra-pulmonary tuberculosis 1. Tuberculosis of bones and joints accounts for 35% of all extrapulmonary tuberculosis cases and most often involves the spine of about 50% of all cases of bone tuberculosis. Spinal involvement is usually a result of hematogenous spread of the pulmonary lesion or from infection of the genitourinary system 2.

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Spinal involvement differs in both children and adults because the adult spine is already static because it is not in the growth phase, whereas the spine in the child is dynamic which means that the child’s bones are cartilaginous. Moreover, in the growth phase, if it is exposed to tuberculous spondylitis, it will have an impact morbidity; thus, it can affect the growth of course. Both conservative and operative management actions have distinct handling and are still controversial.

The average incidence rate of tuberculous spondylitis in children is not known for certain, but it is estimated that 5%−10% of children under 5 years are infected with tuberculosis and half of it occurs in the spine as a secondary manifestation with its primary manifestation usually originating from the lung or urogenital tract. A patient’s history and clinical symptoms are important, but it was not always reliable for early diagnosis.

Neurological deficits in tuberculous spondylitis result from the formation of cold abscesses, granulation tissues, necrotic tissue and sequestrum bone or intervertebral disc tissue and occasionally vascular thrombosis of the spinal artery. Tuberculous spondylitis is a chronic disease and slow to progress with long-lasting symptoms. Management of tuberculous spondylitis is still much difference of opinion between conservative and operative. Some authors recommend the administration of drugs only, while others recommend drugs with surgical intervention. Aggressive decompression, 12 months of anti-tuberculosis drug delivery and spinal stabilization can maximize the preservation of neurological function. However, anti-tuberculosis drugs have a major role in their treatment, but surgical procedures also have their own role in the treatment of tuberculous spondylitis. Conservative treatment cannot prevent the risk of progressivity from kyphotic deformity. Surgery is indicated if there are spinal deformities, neurologic deficits, tuberculosis abscesses and conservative treatment failure. Then, the aim of this study to compared the results of conservative and operative therapy in the management of tuberculous spondylitis in children.

**Method**

This study used an observational analytic research design. The research design used was pre and posted tested controlled group design. Researchers only evaluated the results of conservative therapy and operative action in the management of tuberculous spondylitis in children performed at SMF Orthopedic Dr. Soetomo Teaching Hospital Surabaya. The study was conducted in January 2010 until December 2012.

The sample used was all patients with tuberculous spondylitis who underwent conservative or operative therapy in Dr. Soetomo Teaching Hospital. The samples were divided into two groups. Inclusion criteria included patients aged <18 years old, suffering from tuberculous spondylitis by conservative or operative therapy in Dr. Soetomo Teaching Hospital and willing to participate in this research. The exclusion criteria included no other neuromuscular disease disorders, neurological disorders both central and peripheral, and patients who refused to participate in the study.

The procedure of sampling were patients with tuberculous spondylitis visited to orthopedic unit or patients who were consulted from other inpatients to orthopedic hospitalization at Dr. Soetomo Teaching Hospital Surabaya, the patients underwent clinical, laboratory and radiological examination to diagnose tuberculous spondylitis.

All the data collected were in tabular and statistically processed using SPSS 20.0 (SPSS. Inc. Chicago IL). To compare the pain threshold value (VAS), neurological deficits (Frankle) and Cobb’s angle before and after in conservative therapy and operative action in the management of child tuberculous spondylitis used paired t-test. To compare the change of pain threshold value VAS neurological deficit (Frankle) and Cobb’s angle between conservative therapy and operative action in the management of child tuberculous spondylitis used independent sample t-test and the significance level (α) was set at 0.05. It was statistically significant if p <0.05.

**Result**

**Patient Distribution Based on Treatment Action**

The distribution of conservative and operative patients found that the number of patients was 15 cases with 54% percentage for conservative and 13 operative cases with the percentage of 46% of the total treatment of 28 patients. Its showed that operative action was conducted at one stage of operation. The most action was debridement with bracing installation of 6 cases with 46% percentage. Action with cast, fibular graft and posterior stabilization were 2 cases each with 15% percentage and debridement action with anterior stabilization was 1 case with 7% percentage on table 1.
Patient Distribution Based on Gender and Age

Gender distribution was found that the number of male patients was 16 cases with the percentage of 57% for conservative and 12 operative cases with the percentage of 43% of the total treatment of 28 patients. Then, distribution of patients by age group found that the number of age group of 6–10 years old mostly suffered from tuberculous spondylitis with the amount of 11 cases with percentage of 39%, followed by the number of age group 0–5 years old with the number of 9 cases with the percentage of 32%, age 11–15 years old of 5 cases with percentage 18% and age group 16–18 years old of 3 cases with percentage 11% from total treatment of 28 patients on the table 2.

Patient Distribution Based on Pain Scale (VAS)

Distribution of patients based on the pain scale (VAS) found that most patients with 7–8 pain scale were 12 cases with a percentage of 43%. The pain scale of 4–6 was 10 cases with percentage of 36%, 4 cases with pain scale of 9–10 with percentage of 13% and 1 case each with pain scale of 0–1 and 2–3 with percentage of 4% (Table 2). From 28 subjects, 15 cases were categorized as conservative therapy groups and 13 cases were categorized as operative group (Table 1).

Table 1. Group Distribution

<table>
<thead>
<tr>
<th>Group Distribution</th>
<th>Conservative</th>
<th>Operative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual Analog Scale</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 – 1</td>
<td>1 (7%)</td>
<td>0</td>
</tr>
<tr>
<td>2 – 3</td>
<td>0</td>
<td>1 (8%)</td>
</tr>
<tr>
<td>4 – 6</td>
<td>6 (40%)</td>
<td>4 (31%)</td>
</tr>
<tr>
<td>7 – 8</td>
<td>7 (46%)</td>
<td>5 (38%)</td>
</tr>
<tr>
<td>9 – 10</td>
<td>1 (7%)</td>
<td>3 (23%)</td>
</tr>
<tr>
<td>Frankle Classification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>1 (7%)</td>
<td>4 (31%)</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>6 (40%)</td>
<td>3 (23%)</td>
</tr>
<tr>
<td>D</td>
<td>7 (46%)</td>
<td>2 (15%)</td>
</tr>
<tr>
<td>E</td>
<td>1 (7%)</td>
<td>4 (31%)</td>
</tr>
<tr>
<td>Cobb’s Angle (Kaplan Classification)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild &lt;300</td>
<td>6 (40%)</td>
<td>4 (31%)</td>
</tr>
<tr>
<td>Severe 30 – 600</td>
<td>7 (47%)</td>
<td>6 (46%)</td>
</tr>
<tr>
<td>Moderate &gt;600</td>
<td>2 (13%)</td>
<td>3 (23%)</td>
</tr>
</tbody>
</table>

From that table, it showed, distribution of patients based on neurological deficits found that patients with no neurological deficit (Frankle E) were 11 cases with a percentage of 39%. There were 17 cases with neurological deficit (Frankle A, B, C, and D) with details of 8 cases of Frankle D with percentage of 29%, 4 cases of Frankle C with percentage of 14% and 5 cases of Frankle A with percentage of 18%. From 28 cases evaluated, 15 cases were categorized as conservative therapy group and 13 cases were grouped as operative group (Table 1).
**Patient Distribution Based on Cobb’s Angle**

Distribution of patients based on neurological deficits found that patients with no neurological deficit (Frankle E) were 11 cases with a percentage of 39%. There were 17 cases with neurological deficit (Frankle A, B, C and D) with details of 8 cases of Frankle D with a percentage of 29%, 4 cases of Frankle C with a percentage of 14% and 5 cases of Frankle A with a percentage of 18%.

**Infected Vertebrae Characteristic**

Distribution of patients based on the affected vertebrae level was found that the thoracic and lumbar vertebrae sites were most affected by tuberculous spondylitis with an amount of 8 cases each with a percentage of 28.6%, followed by thoracolumbar and lumbosacral levels of each 4 cases with the percentage of 14.3%, while the cervicothoracic level area with the number of 3 cases with the percentage of 10.7% and the cervical area of 1 case with 3.6% percentage and not found one case in the sacral area of the total treatment of 28 patients (Table 1).

Characteristics of patients studied based on gender, age and level of pathological vertebrae, found that patients who received operative and conservative therapy were not significantly different in terms of gender ($p = 0.477$), age at diagnosis ($p = 0.756$), age at study ($p = 0.788$), and pathology level ($p = 0.539$). Due to the uniform sample, the analysis of the patient’s surgical or conservative results was valid for comparison (Table 2).

**Table 2. Research Analysis**

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Therapy</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics of Research Sample</td>
<td>Conservative (n=15)</td>
<td>Operative (n=13)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>10 (66.7)</td>
<td>6 (46.2)</td>
</tr>
<tr>
<td>Female</td>
<td>5 (33.3)</td>
<td>7 (53.8)</td>
</tr>
<tr>
<td>Age at diagnosis (years)</td>
<td>8.0±5.3</td>
<td>7.5±3.9</td>
</tr>
<tr>
<td>Present Age (year)</td>
<td>9.3±5.3</td>
<td>8.8±4.3</td>
</tr>
<tr>
<td>Location</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervical</td>
<td>2 (11.8)</td>
<td>2 (20.0)</td>
</tr>
<tr>
<td>Thoracal</td>
<td>6 (35.3)</td>
<td>5 (50.0)</td>
</tr>
<tr>
<td>Lumbal</td>
<td>9 (52.9)</td>
<td>3 (30.0)</td>
</tr>
<tr>
<td>Level</td>
<td>3.2±1.4</td>
<td>3.5±1.5</td>
</tr>
<tr>
<td>Characteristics of VAS, Frankle and</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cobb’s Angle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAS</td>
<td>7 (1 - 9)</td>
<td>7 (3 - 9)</td>
</tr>
<tr>
<td>Frankle</td>
<td>4 (1 - 5)</td>
<td>3 (1 - 5)</td>
</tr>
<tr>
<td>Cobb’s Angle</td>
<td>38.7±26.3</td>
<td>46.1±35.9</td>
</tr>
<tr>
<td>After treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAS</td>
<td>-4 (-8 – (-1))</td>
<td>-5 (-8 – (-1))</td>
</tr>
<tr>
<td>Frankle</td>
<td>0 (0 - 1)</td>
<td>1 (0 - 4)</td>
</tr>
<tr>
<td>Cobb’s Angle</td>
<td>6.5±8.0</td>
<td>-3.4±21.1</td>
</tr>
</tbody>
</table>
From table 2, it was found that before the treatment of VAS and Frankle, there was no significant difference in patients’ condition (p = 0.489 and p = 0.091). The patients’ VAS who received the same medication therapy was the same as those who received conservative therapy.

In other side, age, location and number of affected segment vertebrae were not correlated with outcomes to visual analog scale (VAS), frankle improvement and Cobb’s angle. Its show on table 3.

### Table 3. The Correlation Analysis between Age, Location and Number of Segments with Outcome (Visual Analogue Scale, Frankle Improvement and Cobb’s Angle)

<table>
<thead>
<tr>
<th>Variable</th>
<th>VAS (n=28)</th>
<th></th>
<th></th>
<th>Frankie (n=28)</th>
<th></th>
<th></th>
<th></th>
<th>Cobb’s(n=28)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pearson</td>
<td>Sig (2-tailed)</td>
<td>Pearson</td>
<td>Sig (2-tailed)</td>
<td>Pearson</td>
<td>Sig (2-tailed)</td>
<td>Pearson</td>
<td>Sig (2-tailed)</td>
<td>Pearson</td>
<td>Sig (2-tailed)</td>
</tr>
<tr>
<td>Age</td>
<td>-0.057</td>
<td>0.772</td>
<td>-0.039</td>
<td>0.842</td>
<td>0.077</td>
<td>0.698</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Segments</td>
<td>-0.033</td>
<td>0.867</td>
<td>0.028</td>
<td>0.888</td>
<td>0.132</td>
<td>0.504</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location</td>
<td>-0.345</td>
<td>0.072</td>
<td>-0.362</td>
<td>0.058</td>
<td>-0.237</td>
<td>0.225</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Correlation is significant at the 0.01 level (2-tailed)

From the table there is correlation that significant about age, location, and number. There is some segments that give outcome. Pathology affected by 2 segments upwards may make the cut of point for operative action (Table 3).

There was a significant difference in both the conservative and operative group of subjects in which subjects who underwent operative therapy experienced significant pain relief compared to subjects who underwent conservative therapy. This is consistent with several studies conducted in Indonesia. Another result stated that pain can be healed in tuberculous spondylitis which is undergoing surgery. Pain in the tuberculous spondylitis decreased at week four after undergoing operative therapy with debridement.

Comparative assessment of conservative and operative therapy in this study used a pain scale assessment with VAS, neurological deficits assessed by the Frankle classification and assessment of destruction of corpus vertebrae damage using Cobb’s angle. Assessment of the pain scale corresponds to the most frequent complaints in children who suffer from tuberculous spondylitis in daily life such as during sleep, sitting, standing and walking. Assessment of neurological deficits using the Frankle classification scale assessed by sensory and motor examination is also common in children with tuberculous spondylitis.

Not all tuberculous spondylitis has a neurological deficit. Neurological deficits can occur because of the active phase of the inflammatory edema, extra-dural compression to the posterior side of the abscess (pus, the caseous material/granulation, connective tissue granulation, sequester), the gibbus due to collapse of the infected vertebrae. Neurological deficits can’t be recovered due to spinal stenosis, direct compression of the gibbus itself or fibrosis contraction from epidural.

The pathology of tuberculous spondylitis is anterior to the destructive vertebral corpus. This destruction will cause the collapse of the anterior portion of the corpus vertebrae resulting in a sharp angle known as gibbus. Cobb’s angle assessment is important because it can be one of the prognostic factors in children. Moreover, it is appropriate to say that kyphosis is more common in children than in adults because its deformity in children is dynamic with progressive variation through growth, whereas in adults the deformity of kyphosis is static and it depends on the number of levels of the destructive vertebra. This is also supported by previous studies which suggest that conservative-treated tuberculosis spondylitis increases the deformity of kyphosis 10–150 with late kyphosis deformity.
Previous studies have suggested that the status of the posterior column and the type of fixation is a major factor in determining this kyphosis deformity. Kaplan’s classification method for assessing the severity of this Cobb’s angle is mild with angle <30°, moderate with angle of 30–60° and severe with angle >60°. Tuberculous spondylitis with severe kyphosis is very difficult to return to the normal value of kyphosis and required an operative reconstruction after healing process. Other studies suggest that at Cobb’s angle of >60°, anterior decompression action, shortening posterior and stabilization with posterior instrumentation are required. In this study, there are differences in subjects who receive conservative and operative therapy if it is evaluated based on evaluation of pain, neurological deficit and Cobb’s angle. The results of this study provide information on the importance of operative role in the treatment of tuberculous spondylitis.

**Conclusion**

The operative action gives better results than the operative actions of the assessment of visual analog scale (VAS), Frankle and Cobb’s angle. There was no correlation/difference in age, location and number of infected segments with conservative and operative action based on visual analytical scale (VAS) measurement, Frankle and Cobb’s angle.

**Conflict of Interest:** Nil

**Source of Funding:** Self

**Ethical Clearance:** This study was approved by Ethical Commission of Health Research Faculty of Medicine University of Airlangga.

**References**


A New Automated Approach for Early Lung Cancer Detection with Improved Diagnostic Performance – A Preliminary Analysis

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Abstract—Lung cancer is becoming the major cause of cancer-related deaths in human worldwide and Saudi Arabia is not an exception. Therein the identification of potentially malignant lung nodules is essential for the diagnosis and clinical management of lung cancer. Unfortunately, in clinical practice, however, interpretation of Computed Tomography (CT) images is challenging for radiologists due to the large number of cases. It is therefore extremely important task to develop computer aided diagnosis (CAD) systems that can aid and enhance the radiologist to potentially reduce false positive (FP) findings. Even though numerous methodologies are proposed for CAD system in the literatures, the one proposed in this work will definitely stand out in improving the sensitivity and specificity for the detection of small nodules particularly in low dose CT images. This work attempts to employ the powerful tool, radiomics quantitative imaging features within curvelet domain to detect and characterize lung nodules with improved sensitivity and specificity. Subsequently, support vector machine (SVM) is used to learn the 2D stochastic and 3D anatomic features of curvelet coefficients and classifies suspected regions either as malignant or benign based on geometric, texture and intensity. A preliminary analysis of the proposed methodology is presented and compared against the metrics, sensitivity, specificity and accuracy on publicly available LIDC database to serve as a benchmark for future research efforts.

Keywords: Medical image Processing, Low-Dose Computed Tomography, Lung Cancer Detection, Computer-Aided Detection, Anisotropic Diffusion, Radiomic Image Features, Curvelet Transform and Support vector machine

Introduction

Lung cancer stands out among all other types of cancer with highest incidence rates and highest mortality rates in human worldwide. The prevalence is steadily escalating and alarming due to adoption of western lifestyle habits. Also, it is expected that in coming years lung cancer will represent a major public health problems. In practice, Lung cancers are reported at advanced stages and this dramatically diminishes the potential success of treatment and results in the death of around 50% of cancer patients within five years of the late diagnosis. If lung cancer is detected at its earliest stage, the five-year survival rate can reach 70%. These figures call for effective technique to detect lung cancer at early stage to reduce the mortality rate of the patient and improve their life expectancy.

In lung cancer research, CT has become the most sensitive imaging modality for the detection of pulmonary nodules, particularly since the introduction of helical multi-slice technology. In current clinical practice, Low-Dose CT is adopted for Lung Cancer screening to avoid the risks of ionizing radiation associated with CT. Unfortunately, the interpretation of Low-Dose CT images is challenging for radiologists to distinguish between benign and malignant nodules. Added to, the large number of cases and manual reading may cause error-prone and the reader may miss nodules and thus a potential cancer. Thus, there is a pressing need for the use of CAD systems that can aid/enhance radiologist workflow and potentially improve nodules detection.

Current CAD systems often only allow radiologists to visually describe the tumors or lesions and thus are limited to subjective and qualitative characterizations of
the tumors. This is because the algorithms employed in current CAD have high sensitivity that some non-nodule structures (e.g., blood vessels) are labelled as nodules inevitably in the initial nodule identification step. Since the radiologists must examine each identified object, it is highly desirable to eliminate these FPs as much as possible while retaining the true positives (TPs). Thus, it is very time-consuming for radiologists to perform extensive review of all available imaging data. As such, there is significant potential for improving diagnostic accuracy and efficiency through the use of more objective and quantitative approaches for tumor characterization.

One of the biggest emerging areas in recent years related to quantitative cancer screening and diagnosis is radiomics, which involves the high-throughput extraction and analysis of a large number of imaging-based features for quantitative characterization and analysis of tumor. The use of radiomics-driven approaches allows for a more objective and quantitative evaluation and diagnosis of cancer, which can significantly reduce inter-observer and intra-observer variability and improve diagnostic accuracy and efficiency compared to current qualitative cancer assessment strategies. A number of studies have shown that radiomics can be used to characterize tumors and can have clinical significance towards diagnosis.

Despite its potential for huge clinical impact, the current state-of-the-art radiomics techniques make use of pre-defined, hand-engineered imaging-based feature models based on texture, shape, and intensity, which can limit its potential for fully characterizing the unique traits and characteristics of lung cancer lesions as they are still largely based on specific visual traits that radiologists use for subjective interpretation. Therefore, an effective way that can identify and extract imaging features automatically compared to pre-defined and hand-engineered imaging feature models is expected to have potential in capturing tumor phenotype and improve lung cancer detection. Recently, Shen et al. and Kumar et al. have shown the feasibility of discovering radiomics using a deep convolutional neural network learning rather than using predefined, hand-engineered feature models. The main disadvantage of neural network methods lies in training process to adjust the network weights by supervised learning with standard training data. Secondly, the model performance is based on the large dataset employed for training process. Not only that, for each image in the training set, nodule structure needs to be precisely marked by a radiologist is mandatory process.

In this essence, the proposed work first investigates the association between radiomic features based on curvelet transform and lung tumor characterization. Next it employs machine-learning method, SVM to build radiomics-based multivariate classifiers for tumor characterization. Non-invasive and cost-effective radiomic data is expected to improve the histological classification and hence the treatment/thrapy, which in general could have a large impact in cancer care. Thus, the work here will serve as a promising prognostic tool for informing treatment choice and fostering therapy for lung cancer patients.

Proposed Methodology

Proposed Methodology

Considering and keeping in mind the detail survey review reported, the research methods for the proposed CAD system are devised with new efforts to utilize the effectiveness of radiomic feature model approach within curvelet domain as follows:

A. Image Processing

In practice, noise is the common problem in CT imaging under low dose protocol. Lot of studies have shown that noise on CT images is found to be Gaussian. Such degradations have a significant impact on the image quality and as a result, it affects the accuracy of CAD system even the experts with sufficient experience are not be able to draw correct and useful information from the images. Additionally, feature extraction, analysis, recognition and quantitative measurements become difficult and unreliable due to poor quality of images. Thus, the denoising and enhancement of the medical images become prime requirements for many practical applications.

From literature studies, it was clear that though several studies are based on noise filtering in the wavelet domain, they fail to give better results in edgy region because of generation of large wavelet coefficients even at fine scales and repeated at scale after scale, for the edges in the image. On the other hand, the anisotropic feature having the expertise particularly in preservation of edgy region in the denoising process will be considered to suppress noise and lung image enhancement. To improve the speed, the iterations of the diffusion filters are kept small but the diffusion factor is optimized with the trade-off between noise reduction and feature preservation.
B. ROI Extraction

Many of the earlier works employs predetermined threshold value to separate the lung from the surrounding anatomy. Nevertheless, there are also some works that employ optimal thresholding for this task. For example, Otsu’s thresholding method is based on the idea of finding a threshold value that minimizes the within-class variance of resulting foreground and background classes\(^{15}\). The method is robust, and it gives reasonable thresholding results in a vast variety of cases. However, this method has been shown to break down for a certain range of object-to-background pixel population ratios. With respect to this point, the current work refined Otsu’s thresholding employing fuzzy membership function. Subsequently, the edges of lung are restored to prevent the exclusion of peripheral nodules by applying rolling ball technique described by Silva Sousa et al\(^{16}\).

C. Pattern Recognition

Feature extraction is a significant step in the development of an automated characterization of lung nodules. Therefore, in this work, radiomic features are extracted using curvelets proposed by E. Candes and D. Donoho\(^{17}\). Curvelets are waveforms designed as an alternative to the widespread wavelet transforms. Unlike wavelets, has varying degree of localization in orientation with respect to the scale. This property makes curvelet transform capable of multi-scale representation in many directions and positions at each length scale. Also, curvelet takes less number of coefficients for handling discontinuities\(^{18}\). These features of curvelets motivate the usage of curvelet in this work with an objective to efficiently capture the finest curves within the textured medical image with respect to different scales and orientations. In the proposed system, radiomics features includes tumor intensity histogram (e.g., high or low contrast), tumor shape (e.g., round or spiculated), texture patterns (e.g., homogeneous or heterogeneous), as well as descriptors of tumor location and relations with the surrounding tissues (e.g., near the heart). Each of these features will be calculated for three levels of resolution and for each curvelet matrix based on each radial ‘wedge’ (16 angles).

To reduce the dimensionality of the feature space and further reduce the FP rates, SVM is applied in this work to analyze the feature variance and select the relevant features. The SVM finds hyperplane that separates various classes of nodules using Support Vectors\(^{19}\). These support vectors are transformed to a feature space with a nonlinear transformation. The non-linear transformations are represented with kernel function. The kernel function is used to map the input space to feature space. Here, the Morlet Wavelet kernel\(^{19}\) given below, is used as kernel of SVM Classifier

\[
K(x) = \cos(1.75x) \cdot e^{\left(\frac{x^2}{2}\right)}
\]

D. Evaluation Metrics

The accuracy, sensitivity, specificity, and area under the receiver operating characteristic (ROC) curve\(^{22}\) will be employed to as validation metrics to evaluate the performance of the classifier. Here, the sensitivity (true positive rate, TPR) represents the number of true positives divided by the total number of positive cases. The specificity to measure the number of false positive rate. The accuracy (ACC) is the proportion of true results in the population. Finally, the ROC curve to visually illustrate the sensitivity and FP rate for different threshold values.
Figure 1 Illustrates lung nodule detection process on slice number 45 of patient ID LIDC-IDRI-0191. (a) Original DCOM Image (b) Enhanced Image using diffusion filter domain (c) Segmented Binary Lung Image using Fuzzy K-means (d) Binary Image with lung boundary corrected using Rolling ball technique (e) boundary delineating the lung in Original Image (f) Segmented Lung Image (g) Lung Nodule

Experimental Design for Preliminary Study

A preliminary study was conducted to validate the feasibility of the proposed system adopting the publicly available LIDC database at in the National Biomedical Imaging Archive (NBIA)20. This database contains a greater number of subtle and irregularly shaped nodules. Also, the database contains ground truth information obtained from multiple radiologists. This dataset is composed of 1010 patients CT exams with 1500 nodules. LIDC case LIDC-IDRI-0001 through LIDC case LIDC-IDRI-0150 was considered to train the proposed CAD system. LIDC case LIDC-IDRI-0175 through LIDC case LIDC-IDRI-0200 was considered to test the proposed CAD system. During the experiments, the selected training images were also included in the testing stage for the proposed system. The ground truth information annotated by multiple radiologists was used to verify the correctness of the proposed system.

As, reduction of the feature vector is imperative to avoid SVM over-fitting and to obtain a manageable feature space, the preliminary experiments validated the system for several resolution levels and support vector kernels. Initially, three levels of resolution were investigated. A feature vector averaged over co-occurrence directions (99 total features) was compared
with a feature vector averaged over both co-occurrence directions and curvelet details (33 descriptors). The feature vector averaged over both co-occurrence directions and curvelet details was discovered to be ideal. The following sets of descriptors were calculated: features on individual levels of resolution (1, 2, or 3, each with only 11 descriptors), features based on two levels.

**Results and Discussion**

The results obtained from preliminary experimental settings are shown in Fig. 1 for the patient-id LIDC-IDRI-0191 and slice number 45. Although the preliminary results of our proposed system are encouraging and is expected to improve the specificity and sensitivity in Lung cancer diagnosis, we need more cases to test for generality. We will continue to perform additional testing and training with CT cases comprising nodules of various sizes and shapes available in the image database I-ELCAP. More importantly, extensive performance comparison between proposed system and radiologists will performed to evaluate the role of this system in clinical practice.

Next, since the nodules detected with the proposed CAD system were included in the reference standard, the study results could be potentially biased toward making the proposed CAD appear more accurate than it really is. But at this point, we are unable to confirm or reject the influence of this potential bias based on our preliminary study results. Therein, CT cases with complex nodule structures will be identified and considered for testing. The detection of such abnormal nodules may require a modification of current CAD implementation or an introduction of new algorithms or fine-tuning of parameters for the current system.

In future work, focus of attention will be put to improve the clinically relevant performance measures, sensitivity-specificity calculus of the proposed CAD systems. In this regard, systematic study will be performed to assess the relative weights and valid ranges of parameter values on the detection performance of the proposed system. We have planned to evaluate the ability of the classifier in the task of differentiating nodule candidates that correspond to actual nodules from false-positive candidates.

**Ethical Clearance:** The proposed work is a retrospective study that uses images from the public database archives. Therefore, there was no requirement to obtain any Informed consent from the institutional review boards.

**Source of Funding:** Self

**Conflict of Interest:** Nil

**References**


Validity of Star Excursion Balance Test as Dynamic Balance Test In Subjects with Non-Operative Anterior Cruciate Ligament Injury

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Abstract

Background: The anterior cruciate ligament (ACL) injury is the most common knee injury that might occur during exercise. An ACL causes a balance disorder that can be considered as a risk factor for recurring injury. Identification of balance disturbances in subjects with ACL injury should be conducted to minimize the risk of re-injury.

Objectives: this study aims to determine the validity of star excursion balance test with biodex balance system SD as a gold standard

Method: This study is a diagnostic test with cross-sectional study approach. The number of research sample was 20 respondents selected as control group, five people with ACL injury as the case group. All subjects were examined with the biodex balance system SD (Overall Stability Index) and star excursion balance test (% MAXD).

Results: The result of the correlation analysis between the overall stability index and %MAXD was not significant with r = -0.316; p = 0.175. Hence, the cut-off point could not be determined due to the insignificant correlation, thus the sensitivity, specificity, positive predictive value, and negative predictive value of the star excursion balance test could not be determined.

Conclusion: Star excursion balance test was invalid as a dynamic balance test if the Biodex Balance Test SD was used as a gold standard.

Keywords: validity, overal stability index, %MAXD, anterior cruciate ligament injury

Introduction

Sports injuries are the most common type of injury. Researches have stated that the number of sports injuries make up about 21% of the injury cases in the world. Sports injuries can lead to long-term disability or dysfunction especially in patients with knee injuries. The most often structures involved are the anterior cruciate ligament (ACL) injuries in 20% of the knee injury incident, and 46% of the knee ligament injury incidences. An anterior cruciate ligament injury results in knee instability, which inhibits an athlete from playing in the field and can lead to early retirement ¹,².

The anterior cruciate ligament injury causes a proprioceptive disorder and it is suspected to be caused by mechanocarpeptic damage to the joints and ligaments. Sensory input from proprioceptive, vestibular, and visual plays a role in maintaining static or dynamic balance. Proprioceptive, vestibular, and visually impairment can lead to a imbalance in the body. Identification of a balance disorder in subjects with ACL injury should be performed to minimize the risk of re-injury ¹–³.

Dynamic balance plays a pivotal role as the athlete moves and reacts quickly to the conditions...
that occur in a sports game. Athletes often experience a perturbation that disturbs his balance either from an opposing player or from himself as he changes position and direction. The perturbation is strong and requires stability. Athletes with a good balance have superior performance compared to ordinary people. Dynamic balance disturbances have been reported as a result of injury to athletes. Standardized assessment to measure and evaluate balance is needed to determine an athlete’s readiness to return in the field 4,5.

The identification of balance disorders can be assessed in various ways, i.e., clinically, by tools, or by using a valid and reliable scale. Objective examination to determine the condition of balance can be done by using biodex balance system SD. This tool can be used to assess the balance in static format and semi-dynamic. Stability index (SI) is obtained from the dynamic balance test to determine how well the patient controls their balance in both anterior/posterior (A/P) and medial/lateral (M/L) directions. Balance assessment is expressed by overall stability index (OSI). Biodex balance system SD is a valid and reliable tool for assessing dynamic balance6,7.

The most common test used for balancing in athletes is the star excursion balance test (SEBT). This test requires balance on one foot of support (single leg stance) to achieve the maximum range that can be reached by the opposite leg. The distance that can be reached by the participants is an index of dynamic balance. The further the distance, then the balance control is also better. The mean value of the reach distance (% MAXD) is a description of the dynamic balance value. Star excursion balance sensitive test identifies a reach distance deficit as a dynamic balance in subjects with chronic ankle stability, patellofemoral pain syndrome, and anterior cruciate ligament deficiency. Implementation of this test uses a simple tool, thus it can be done anywhere. SEBT is a potential option as a cost-effective tool and reliable to assess the balance deficit in various pathological conditions of the lower limbs 8. A study to assess the validity of SEBT in assessing dynamic balance has never been conducted before. Thus the researchers aim to determine the validity of star excursion balance test with biodex balance system SD as a gold standard.

**Method**

This research is an observational analytic study with cross-sectional study approach. The study was performed at the Rehabilitation Installation Unit of Universitas Airlangga Hospital Surabaya from July to October 2014. The sample unit was subjects with ACL injury that fulfilled the inclusion criteria (male patients with unilateral ACL injury for >6 weeks at the age of 17-30 years, having a minimum exercise routine of three times a week, able to understand and follow the instructions, and willing to participate in this research by signing an informed consent).

The subjects consisted of five patients with unilateral anterior ACL injuries and five healthy people as the control group, thus we obtained 20 respondents for the sample units. The balance assessment was performed with the biodex balance system SD and star excursion balance test. The examination was finished in a day. The protocol of this study was approved by Dr. Soetomo Teaching Hospital, Surabaya, Indonesia. Data analysis was performed using Pearson correlation test with SPSS for Windows version 17 (SPSS, Inc., Chicago, IL).

**Results**

**General Characteristics of Subjects**

The general characteristics of the subjects were shown in Table 1. The average age of the subjects in the control group was 26.60±3.24 years, and 23.20±3.73 years in the case group. The average height of the controls was 173.60±5.72 centimeters, and the average height of the subject with ACL injury was 169.40±5.27. The average weight of the control group was 70.40±11.17 kilograms and 65.00±5.37 kilogram on the subjects with ACL injury. The mean body mass index of the controls was 23.26±2.67 kg/m² and 22.74±2.56 kg/m² on subjects with ACL injury.
Table 1. General Characteristics of Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Groups</th>
<th>Minimal</th>
<th>Maximal</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
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<td>Control</td>
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<td>30</td>
<td>26.60±3.24</td>
</tr>
<tr>
<td></td>
<td>Case</td>
<td>20</td>
<td>30</td>
<td>23.20±3.73</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>Control</td>
<td>167</td>
<td>181</td>
<td>173.60±5.72</td>
</tr>
<tr>
<td></td>
<td>Case</td>
<td>163</td>
<td>171</td>
<td>169.40±5.27</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>Control</td>
<td>63</td>
<td>86</td>
<td>70.40±11.17</td>
</tr>
<tr>
<td></td>
<td>Case</td>
<td>57</td>
<td>70</td>
<td>65.00±5.37</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>Control</td>
<td>19.0</td>
<td>26.3</td>
<td>23.26±2.67</td>
</tr>
<tr>
<td></td>
<td>Case</td>
<td>18.0</td>
<td>24.7</td>
<td>22.74±2.56</td>
</tr>
</tbody>
</table>

The Results of Overall Stability Index and %MAXD

The balance of the subjects that was measured using the biodex balance system SD produced the overall stability index (OSI) on both limbs of control and case groups. The results of OSI and %MAXD are shown in table 2.

Table 2. Examination Results of Overall Stability Index and % MAXD

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Minimal</th>
<th>Maximal</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSI</td>
<td>Control</td>
<td>0.4</td>
<td>0.7</td>
<td>0.550±0.097</td>
</tr>
<tr>
<td></td>
<td>Case</td>
<td>0.5</td>
<td>1.2</td>
<td>0.786±0.286</td>
</tr>
<tr>
<td>% MAXD</td>
<td>Control</td>
<td>0.76</td>
<td>0.98</td>
<td>0.863±0.901</td>
</tr>
<tr>
<td></td>
<td>Case</td>
<td>0.66</td>
<td>0.83</td>
<td>0.772±0.076</td>
</tr>
</tbody>
</table>

OSI: overall stability index

MAXD: maximum distance

The mean of OSI in the control group was 0.55±0.097, with an OSI. The mean OSI on the injured subjects was 0.786±0.286. The balance of the subjects measured using the Star excursion balance test on both limbs of control and case groups resulted in %MAXD. The mean of %MAXD in the control group was 0.863±0.901 with a %MAXD. The mean of %MAXD in the case group was 0.772±0.076.

The Association of Overall Stability Index with %MAXD

The association of OSI with %MAXD was assessed using Pearson correlation test. Correlation analysis of OSI with %MAXD was not significant with r = -0.316, p = 0.175 (table 3.).

Table 3. Association of OSI with % MAXD

<table>
<thead>
<tr>
<th>Test</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson correlation</td>
<td>-0.316</td>
<td>0.175</td>
</tr>
</tbody>
</table>

The cut-off point could not be determined due to the insignificant correlation, thus the sensitivity, specificity, positive predictive value, and negative predictive value of the star excursion balance test could not be determined.
The Difference of Overall Stability Index and %MAXD in Subjects with Anterior Cruciate Ligament Injury and Control Group

Overall Stability Index in control and case groups was tested using T-test, the difference was significant with \( p = 0.031 \). The% MAXD score in the control and case groups was analyzed using T-test, thus a significant difference was found with \( p = 0.025 \).

Discussion

This study applied the postural stability test protocol and a single leg stance. The normal value of OSI on subjects with the age of 17-35 years was 0.82-2.26. The OSI range below the upper limit of normal values in healthy subjects ranged from 0.4 to 0.7 and from 0.5 to 1.2 in the subjects with an injury. These findings showed that the normal range of values that was used could not distinguish the presence of a balance disorder in the case group. This was likely to occur because the normal values used were determined by the normal population at the age of 17-35 years. The subjects of the study were physically trained people, thus the physical performance was better than normal people who did not practice any regular physical exercise. Therefore, the OSI values obtained were smaller than the normal range of values that already existed. Determination of the OSI normal range in the physically trained subjects as in the athlete was necessary in order to detect balance disorders using BBS.

The balance of the subjects was measured using the star excursion balance test on both limbs of control and case groups that resulted in %MAXD. The %MAXD range in the control group ranged from 0.76 to 0.98, while it ranged from 0.66 to 0.83 in the case group. The cut-off point of %MAXD could be determined if there was a significant correlation with the value of the gold standard. The reference value of the overall stability index (OSI) as measured by the current biodex balance system could not be used to set subjects with high activity levels or athletes as the gold standard of balance. This is due to the absence of a normal range of OSI values in the athlete population or subjects with high activity level. Thus, this study could not determine the cut-off point of % MAXD. Studies to determine the normal range of OSI values in a population of physically trained subjects such as athletes were required to be used as a reference in terms of the balance value in the population. The study to determine the normal value of %MAXD has never been performed, however, the assessment of the normal range of %MAXD component has been done previously, covering the distance assessment on anterior, anterolateral, anteroposterior, posterior, posteromedial, medial, and anteromedial. Research to determine the reliability needs to be performed before the study to determine the normal value of %MAXD in the athlete population was performed.

There was no significant correlation between OSI with %MAXD; OSI showed semi/dynamic balance, meanwhile, MAXD showed dynamic balance. The results of this study differed from the research by Bakhtiari (2012) that a significant correlation was found between static balance as assessed by flamingo balance test and dynamic balance assessed by SEBT.

The OSI assessment was obtained from an examination with the biodex balance system SD (BBS), the subjects were asked to stand on one foot on a fixed platform then maintained a single-leg stance while adjusting the position of the balance point drawn on the screen. There was no change in the center of gravity and in a base of support, thus the balance assessed using BBS was a static balance. In contrast to SEBT, subjects were required to stand on the limbs, while the contralateral limbs tried to reach the range as far as possible in the prescribed direction and then returned to the starting point by maintaining a balance on the limb of the pedestal. The test described the dynamic balance of the ability to maintain a balance when the body performed a functional movement.

Static balance control is required to maintain anti-gravity positions such as standing or sitting whereas dynamic balance is necessary to stabilize the body when the surface while the body rests or when the body moves on a stable surface. There might be sudden changes (perturbation) of balance when a person is currently conducting an activity, thus a balance disorders occurs. When a perturbation happens in healthy people, then the body reacts with a variety of mechanisms in order to maintain the balance, it depends on the direction and magnitude of perturbation experienced by the person. If the perturbation is small or occurs slowly or when standing still, then the ankle creates a movement in an effort to center the mass in a stable position. If the perturbation occurs suddenly and strong or while performing the movement, thus the hip is used to maintain the balance. Previous research reported that inappropriate static balance assessments were used...
to assess dynamic balance in the absence of movement at the center of gravity due to the response of muscular activity 12. However, this study found no significant correlation between OSI and %MAXD. The absence of significant correlation caused the cut-off point of SEBT to not be able to be determined. Therefore, the sensitivity, specificity, positive predictive value, and negative predictive value of star excursion balance test could not be determined.

The star excursion balance test is considered sensitive in detecting deficits of dynamic balance in patients with leg injuries such as chronic ankle instability (CAI), ACL injury, and patellofemoral pain syndrome 8. There was no significant difference between the normalized reach distance between the control and case groups. The %MAXD score describing the dynamic balance in the subjects with ACL injury was lower than the control group in this study, there was a significant difference with p = 0.025. Significant differences of balance index in subjects with ACL injury were obtained from the research by Herrington (2009) 2 compared to the control group.

The study by Chen et al (2011) 13 compared the balance of 15 patients with CAI with the control group using SEBT. The reach distance of the control group was greater than the subjects with CAI. Another study found that subjects with CAI achieved a significantly smaller reach distance when resting on injured limbs compared to non-injured limbs as well as other subjects without CAI 2,13,14.

Significant differences were also found in the overall stability index score using the Biodex Balance System SD, where the OSI values of subjects with ACL injury were higher than the control group. This illustrates that the balance deficit in subjects with ACL injury could be identified with the biodex balance system SD tool and could be determined by star excursion balance scale.

**Conclusion**

Star excursion balance test is invalid to be used as a dynamic balance test when biodex balance system SD is set as the gold standard, in which star excursion balance test cannot be used to replace the biodex balance system SD.

**Ethical Clearance:** The study protocol was approved by the Ethical Commission to conduct basic science/clinical research in Dr. Soetomo General Hospital Surabaya, Indonesia. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, non-maleficence, and justice.

**Conflict of Interest:** The author reports no conflict of interest of this work.

**Source of Funding:** This study is done with individual funding.

**References**


Correlation between Parenting Styles and Peer Attachment with Aggressive Behavior Potentials in Adolescent Boys

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Abstract

Background: Adolescence is a transitional phase that causes biological, cognitive, social-emotional, and behavioral changes. One of the issues of concern is aggressive behavior in adolescents. Parenting styles and peer attachment are one of the factors that have an influence on potential aggressive behavior in adolescent boys.

Objectives To analyze the relationship between parenting styles and peer attachment to the potential aggressive behavior of adolescent boys.

Method: The subjects were junior high school male students who are cared for by both biological parents and can follow all of the research procedures. The research design was cross-sectional with total sampling technique. Subjects performed anamnesis related data self, parent demographic data, and peers. (PAQ), (IPPA) (BPAQ). The analyzes used were Pearson, Spearman, and Anova.

Results: The majority of parenting styles was permissive (34.06%; 48.00%). Parenting styles and peer attachments did not have a significant relationship with potential aggressive behavior (p = 0.798; p = 0.071; p = 0.695). There was a negative correlation of peer attachment with alienation dimension (p = 0.001). Other significant dimensions with potential dimensions of aggressive behavior were trust (p = 0.018; 0.022) and communication (p = 0.029; p = 0.000).

Conclusion: Parenting styles and peer attachments did not have a meaningful relationship with potential aggressive behavior in adolescent boys. However, there were some significant correlations between the dimensions of parenting and peer attachment with potential dimensions of aggressive behavior.

Keywords: Peer attachment, Parenting styles, Aggressive behavior potential, Adolescents.

Introduction

Adolescence is a transitional phase from child to adult. This transition leads to biological, cognitive, social-emotional, and behavioral changes. One of the issues of concern is aggressive behavior. Fighting behavior of male students in a year period of about 22.00% in Sweden, 44.00% in the United States, and 76.00% in Israel. In Indonesia, acts of violence and crime committed by adolescents increased from 3 thousand cases (2007) to 4 thousand cases (2009). The level of fights among students also increased from 108 cases (2008) to 327 cases (2014).

Aggressive behavior is one of the external behaviors of adolescents. Adolescent boys are 2.5 times higher in aggressive behavior than girl. Factors related to the emergence of aggressive behavior include the interaction between nature (heredity, biological) and...
nurture (experience with the environment, such as parenting and peer attachment). The interactions of both run continuously and influence each other causing an adjustment or adaptation. Parenting styles is one of the major predictors of child behavior, but there is no definitive and comprehensive theory of how parents shaped child development.

Social attachment with peers also contributes to the emergence of aggressive behavior. There is a positive correlation between peer attachment in the group with intentions of aggressive action in adolescents aged 13-16 years, determined by peer influence and self-selection, i.e., if the adolescent wants to take aggressive action then he will look for peer group which facilitates his actions or so should be. Peer attachment affects life satisfaction and well-being of adolescent.

**Method**

The subjects were the 8th-grade male students in SMP Negeri 1 Yosowilangun and SMP Negeri 1 Randuagung Lumajang Regency that meets the inclusion and exclusion criteria. The study design was cross sectional in May-June 2017. The sampling technique was total sampling with 127 students. To demographic data collection such as age, ethnicity, entering puberty or not, sex education source, presence/absence of abuse history, a form of abusive behavior, substance or drug use and never or not committing a crime. Prior to the identification of the subject first, the researchers conducted a test of ethics (No.135/EC/KEPK/FKUA/2017) in Dr.Soetomo Surabaya, Indonesia.

In the parenting styles variables, the subject will fill in the Parenting Authority Questionnaire (PAQ). This instrument consists of 10 statements measured by 5 points Likert (strongly disagree, disagree, neutral, agree, strongly agree). In his judgment, the total number of points for each type of parenting and mother’s parenting (authoritative, authoritarian, permissive, and combination) will be calculated.

The subjects will also conduct peer attachment examination using Inventory of Parent and Peer Attachment-Peer Version (IPPA) questionnaires. IPPA was a self-report questionnaire with 25 statements and the Likert 5-point answer format was almost never, rarely, sometimes, often, almost always.

**Results**

<table>
<thead>
<tr>
<th>Table 1. Characteristics of Basic Data Parents Subject Research</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables</td>
</tr>
<tr>
<td>Characteristics of Parent Demographics</td>
</tr>
<tr>
<td><strong>Education</strong></td>
</tr>
<tr>
<td>Elementary school</td>
</tr>
<tr>
<td>Junior high school</td>
</tr>
<tr>
<td>Senior high school</td>
</tr>
<tr>
<td>Bachelor degree</td>
</tr>
<tr>
<td><strong>Jobs</strong></td>
</tr>
<tr>
<td>Labor</td>
</tr>
<tr>
<td>Government employees</td>
</tr>
</tbody>
</table>
### Table 1. Characteristics of Basic Data Parents Subject Research

| Private employees | 29.90 | 14.20 |
| Farmer            | 33.10 | 18.20 |
| Unemployee        | 1.60  | 52.00 |
| Others            | 17.30 | 6.30  |

<table>
<thead>
<tr>
<th>Earnings of parents</th>
<th>&lt; 1,000,000</th>
<th>1,000,000–&lt;3,000,000</th>
<th>≥3,000,000</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>29.10</td>
<td>73.20</td>
<td>5.60</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Family history with violent behavior</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>26.80</td>
<td>73.20</td>
</tr>
</tbody>
</table>

### Table 2. Relationship Between Parenting styles of Father and the Potential of Aggressive Behavior in Boys

<table>
<thead>
<tr>
<th>Parenting styles of father</th>
<th>%</th>
<th>Mean ± SD Potential of Aggressive Behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Score</td>
<td>Physical aggression</td>
</tr>
<tr>
<td>Authoritative</td>
<td>25.20</td>
<td>80.8 ± 10.4</td>
</tr>
<tr>
<td>Authoritarian</td>
<td>22.00</td>
<td>79.8 ± 8.5</td>
</tr>
<tr>
<td>Permissive</td>
<td>34.60</td>
<td>80.1 ± 7.7</td>
</tr>
<tr>
<td>Combination</td>
<td>18.10</td>
<td>82.1 ± 10.4</td>
</tr>
<tr>
<td>p-value ANOVA</td>
<td>0.798</td>
<td>0.411</td>
</tr>
</tbody>
</table>
Table 3. Relationship Between Parenting Styles of Mothers and Potential Aggressive Behavior in Male Students

<table>
<thead>
<tr>
<th>Parenting styles of mother</th>
<th>%</th>
<th>Mean ± SD Potential of Aggressive Behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total Score</td>
</tr>
<tr>
<td>Authoritative</td>
<td>18.90</td>
<td>83.7 ± 8.2</td>
</tr>
<tr>
<td>Authoritarian</td>
<td>21.30</td>
<td>81.6 ± 9.2</td>
</tr>
<tr>
<td>Permissive</td>
<td>48.00</td>
<td>78.5 ± 9.0</td>
</tr>
<tr>
<td>Combination</td>
<td>11.80</td>
<td>82.2 ± 9.1</td>
</tr>
<tr>
<td>p-value ANOVA</td>
<td></td>
<td>0.071</td>
</tr>
</tbody>
</table>

Note: The superscript letters (a, b, ab) show significant differences using the LSD double comparator test (p < 0.05)

Table 4. Relationship Between Peer Attachment and the Potential of Aggressive Behavior in Boys

<table>
<thead>
<tr>
<th>Peer attachment</th>
<th>Potential of Aggressive Behavior</th>
<th>Physical Aggression</th>
<th>Verbal aggression</th>
<th>Anger</th>
<th>Hostility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p = 0.695 r = 0.035</td>
<td>p = 0.844 r = -0.018</td>
<td>p = 0.067 r = 0.163</td>
<td>p = 0.061 r = -0.156</td>
<td>p = 0.094 r = 0.149</td>
</tr>
<tr>
<td>Trust</td>
<td>p = 0.155 r = 0.127</td>
<td>p = 0.620 r = 0.044</td>
<td>p = 0.018* r = 0.210</td>
<td>p = 0.465 r = -0.065</td>
<td>p = 0.022* r = 0.203</td>
</tr>
<tr>
<td>Communication</td>
<td>p = 0.060 r = 0.167</td>
<td>p = 0.866 r = -0.015</td>
<td>p = 0.029* r = 0.194</td>
<td>p = 0.726 r = -0.031</td>
<td>p = 0.000* r = 0.322</td>
</tr>
<tr>
<td>Alienation</td>
<td>p = 0.001* r = -0.289</td>
<td>p = 0.322 r = -0.089</td>
<td>p = 0.054 r = -0.171</td>
<td>p = 0.006* r = -0.244</td>
<td>p = 0.001* r = -0.301</td>
</tr>
</tbody>
</table>

*p value<0.05
Characteristics of Parents’s Basic Data

The majority of parent education was primary school with the father of 44.10% and the mother of 52.80%. As for the job, the majority of father was more as farmer (33.10%) and mother as housewives (52.00%). The largest parent’s income was Rp. 1,000,000, - up to less than Rp. 3,000,000, - (55.10%). Then, 26.80% of subjects said to have a family with violent behavior (table 1).

The relationship between Parenting styles with the Potential of Aggressive Behavior

The majority of parenting styles were permissive in the father (34.06%) and mother (48.00%) and other authoritative successive parenting (25.20%; 18.9%), authoritarian (22.00%; 21.3%), and combination (18.1%; 11.8%). The type of parenting in the father (p = 0.798) and the mother (p = 0.071) did not have a significant correlation with the total aggressive behavior potential level. In contrast to the pattern of foster parenting, maternal upbringing has a significant correlation with anger dimensions (p = 0.024) with combined parenting patterns showing higher anger levels than other parenting (Table 2).

The relationship between Peer Attachment and Aggressive Behavior Potential

Peer attachment has no significant correlation with potential aggressive behavior (p = 0.695). Only the alienation dimension has a negative correlation with potential aggressive behavior (p = 0.001). The dimensions of trust and communication have a significant correlation with verbal aggression (p = 0.018; p = 0.029) and hostility (p = 0.022; p = 0.000). The alienation dimension has significant negative correlation with anger (p = 0.006) and hostility (p = 0.001), with weak correlation strength (table 3).

Discussion

Father and mother’s parenting styles do not have a meaningful relationship with potential aggressive behavior. Other studies also have similar conclusions. The role of parenting is only marginal. Sometimes, the child is more easily affected by the mother than by the father. A low affection level in permissive parenting is also a predictor of declining external behavior problems, but increases the risk of internal behavioral problems later on 12.

Peer attachment is not related to potential aggressive behavior. This is in contrast to research in Malaysia, but the same conclusion is that there is a negative correlation between the alienation dimension and the potential for aggressive behavior 6. The dimensions of trust and communication are related to the dimensions of verbal aggression and hostility. This condition can also be explained by the child’s efforts to meet the needs of companionship with peers 13. Thus, although there are trust and communication but aggressive behavior can still occur 14. On the dimensions of verbal aggression associated with teen communication with peers. Although verbal, but the psychological effects on people who experience worse than physical aggression 15. While the alienation dimension is negatively related to anger and hostility. Children with withdrawal have a higher risk for internal behavioral problems that may lead to anxiety and depression 16.

Most of the subjects aged 14 years with Javanese, have been puberty and get sex education via the internet. At the age of 13-15 years was a puberty of men with information media also influential convey information about sex in adolescents 17. The educational and socioeconomic level of the subject’s parents was largely in the lower middle class which is one of the factors to the child’s aggressive behavior 18. Parents who give physical punishment/aggression to children become predictors of aggressive behavior in the future through the formation of aggressive fantasy. The majority of subjects have close friends with between 1-3 people, have a group of friends. The subjects mentioned that the behaviors that followed include involving brawl, damaging goods, violating the rules, and using alcohol/drugs together. The influence of peer groups or peers was very strong in adolescence. Teenagers who are part of a peer group were signs of adaptation and separation with parents as well as replacing the focus of loyalty to friends 19.

Foster parenting for most fathers and mothers is a permissive parenting pattern, which has a pattern of parenting with low responsiveness and demandingness. Permissive parenting patterns can make teenagers grow up as impulsive people, act at will, and relate to other less responsible behaviors 20. The average peer attachment value of the study subjects can be classified at moderate levels almost the same as in previous studies 9. The old attachment theory suggests that there was parental influence in the relationship of a child, but attachment to peers was known to have an effect when the child is in
adolescence \(^{21}\).

The old attachment theory suggests that there was parental influence in the relationship of a child, but attachment to peers was known to have an effect when the child is in adolescence \(^{6}\). Physical aggression is caused by several predictor factors such as gender, low family economic status, low maternal education, and a pattern of abusive care \(^{22}\).

**Conclusion**

Parenting styles and peer attachments did not have a meaningful relationship with potential aggressive behavior in adolescent boys. However, there were some significant correlations between the dimensions of parenting and peer attachment with potential dimensions of aggressive behavior.

**Conflict of Interest:** There is no conflict of interest.

**Source of Funding:** This study is self-funded.

**Ethical Clearance:** This study was approved by Ethical Commission of Health Research Faculty of Medicine University of Airlangga (No.135/EC/KEPK/FKUA/2017) in dr. Soetomo General Hospital Surabaya, Indonesia.

**References**


15. Infante DA, Wigley III CJ. Verbal aggressiveness:


Bacteriological Study of *Klebsiella pneumoniae* Isolated from Burn Patient in Al-Najaf City

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¹College of health & Medical tech. / Kufa, Al-Furat Al-Awsat Technical University, 31003 Al-Kufa, Iraq, ²Department of Biology, Faculty of Science, Kufa University, Iraq

**Abstract**

A total of (60) clinical specimens were collected from patients suffering from various burns during the period from November 2018 to January, 2019. These specimens were collected from patients attending to Al-Furat Al-Awsat burn center during the studied period. All specimens were cultured on the MacConkey agar plates and incubated at 37°C under aerobic condition for 18 - 24 hour. In 60 patients, 36 (60%) were female and 24 (40%) were male. Several morphological, physiological and biochemical tests were made to identify bacterial isolates. Results showed that *K. pneumoniae* constitute 15 isolates (25%) of these isolates.

A collection of 15 *K. pneumoniae* isolates diagnosed by the morphological, cultural and biochemical characters, the identification was confirmed by molecular method for the presence of *magA*. The results showed that only 10 (66.6%) isolates was carrying *magA* which are diagnosed as *K. pneumoniae*. The results of antibiotic sensitivity screening test revealed that the isolates of *K. pneumoniae* exhibited highly sensitivity to Azithromycin, moderate sensitivity for Gentamicin and Imipenem while the findings showed absolute resistant to Erythromycin, highly resistant to Ciprofloxacin, Trimthoprime. According to this result it can considered that Azithromycin as best treatment against *K. pneumoniae*.

**Keyword:** Bacteriological, *Klebsiella pneumonia*, burn patient, clinical specimens and Al-Najaf City

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**Introduction**

Burn wound infections are one of the most important and potentially serious complications that occur in the acute period following injury(1). These wounds are subsequently colonized with microorganisms, including gram-positive bacteria, gram-negative bacteria and yeasts, which derived from the host’s normal flora (gastrointestinal flora, upper respiratory flora) and from the hospital environment (2, 3). The risk of invasive burn wound infection is influenced by the extent and depth of the burn injury, various host factors, and the quantity and virulence of the microbial flora colonizing the wound(4). The common burn wound pathogens are, *Klebsiella spp.*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, which produce a number of virulence factors that are important in the pathogenesis of invasive infection(5,6). *Klebsiella pneumoniae* is widely distributed in the gastrointestinal, urinary, and respiratory tracts of healthy people. It cause opportunistic infections mainly nosocomial infections, it is a common hospital-acquired pathogen causing severe respiratory infections such as pneumonia. Other infections caused by this organism include urinary tract infection, wound infection, abscesses, sepsis, inflammation and diarrhea, most *K. pneumoniae* are hospital associated with a high fatality rate if incorrectly treated. Treatment of *Klebsiella* infections is complicated(7). *K. pneumoniae* have different virulence factors which give the bacteria the ability to invade the host, such as capsular polysaccharide, lipopolysaccharide, serum resistance, siderophore production, fimbriae and other factors such as the production of urea and enterotoxin(8). However, antibiotic resistance properties are the major factor in its pathogenicity that it resists for wide spectrum of antibiotics and specially β-lactam antibiotics. This is due to the prevalence of infections acquired in hospital which led to the orientation of the research on alternative therapies (9). Due to the common occurrence of *Klebsiella spp*. And high virulence that cause in the absence of accurate and early detection them, severe damage may lead to the death of the patient. The aim of this study was to determine the causative microorganisms of burn wound infection and antibiotic sensitivity.
2-Materials & Methods

-Specimens Collection:

A total of (60) clinical specimens were collected from patients suffering from various burns during the period from November 2018 to January, 2019). These specimens were collected from patients attending to middle Euphrates burn center during the studied period. All specimens were cultured on the MacConkey agar plates and incubated at 37°C under aerobic condition for 18 - 24 hour.

-Isolation and Identification of Klebsiella pneumoniae

All specimens were initially cultured on isolation media including MacConkey agar. After incubation of agar media for 24 hr. at 37°C, the suspected colonies of pure cultures were investigated. Then the bacteria were confirmed by an additional biochemical test with VITEK-2 compact system and P.C.R technique.

-Antibiotic Susceptibility Test

The detection of the susceptibility of Klebsiella pneumoniae to a group of antibiotics Kirby-Bauer discs diffusion method was carried out according to (11). A pure culture of previously identified bacteria was prepared by adding a growth from isolated colony to 5 ml of sterile normal saline in a cell density equivalent to turbidity of McFarland tube No. (0.5) which approximately equal to bacterial cells density of 1.5x10⁸ cells/ml. A sterile cotton swab was used to obtain inoculums to be streaked on Blood Muller Hinton Agar medium. The antibiotic discs were placed on the surface of the medium at evenly spaced intervals with flaming sterile forceps. Incubate the plate for 18 hr at 37°C. Antibiotic inhibition zone was measured by using ruler. Zone diameter was compared to standard results being recommended by clinical laboratory standards institute documentations.

-Molecular Study of Klebsiella pneumoniae

-Isolation of Bacterial Chromosomal DNA

Total DNA was extracted from colonies grown on agar plates by boiling method according to (12) with some modifications. One bacterial colony was scraped using sterile toothpick from surface of agar plates and suspended in 40 μl Tris-EDTA buffer. The suspension was heated for 15 min at 100°C followed by 5 min on ice rapidly. The suspension containing DNA was stored at -20°C until used as template for PCR.

-Primers Selection:

All primers in this study were synthesized by Bioneer company (Korea). The sequences of this primer were magA: F5'- CGC CGC AAA TAC GAG AAG TG 3'; R5'- GCA ATC GAA GTG AAG AGT GC -3', the product of this gene 540bp (13).

-The PCR conditions of the magA gene: were the initial denaturation temperature of 94°C for 2min. three thermo-cycler condition (1- denaturation of 94°C for 45sec. 2-an annealing temperature of 52°C for 45sec .3- extension of 72°C for 45sec. The final extension of 5 min at 72°C. This condition used for 35 cycles.

-Agarose Preparation

Agarose was weighted 1g, boiled in 100ml (1X) TBE buffer, left to cool at 50°C and 5 µl of ethidium bromide is added to agarose and poured on preparing tray. Comb was removed after hardening of agarose leaving wells.

TBE (1X) buffer was added to the electrophoresis tank, tray with agarose was immersed in electrophoresis tank. Each well is loaded with 7µl of DNA sample and standard molecular weight of DNA ladder (marker) is loaded in a first well. Electrophoreses run at 80 volt/cm for 1hr. Gel was visualized with UV transilluminator and photographed by using digital Camera (14).

3. The results

-Bacterial Isolation

A total of (60) clinical specimens were collected from patients suffering from various burns during the period from November 2018 to January, 2019). These specimens were collected from patients attending to middle Euphrates burn center during the studied period. All specimens were cultured on the MacConkey agar plates and incubated at 37°C under aerobic condition for 18 - 24 hour.

In 60 patients, 36 (60%) were female and 24 (40%) were male as in figure(1). However, (15) stated that female infection was higher (54.5 %) than male (45.6 %). In addition, (16) viewed that 209 cases were males and 995 cases females, patients were mainly of the group ≥ 60 years. They referred that male patients above the age of 60 years were subjected to greater frequency
of *K. pneumoniae* infection.

![Figure 1: Distribution of infected patients according to sex](image)

*Klebsiella pneumoniae* Isolation and Identification

Clinical and environmental hospital samples were cultured on to MacConkey agar and incubated for 18-24 h at 37°C. All lactose-fermenting isolates were tested by morphologic characteristics and standard biochemical tests according to (10). Confirmation of *K. pneumoniae* was conducted using P.C.R system. Several morphological, physiological and biochemical tests were made to identify bacterial isolates. Results showed that *Klebsiella pneumoniae* constitute 15 isolates (25%) of these isolates, The other bacterial isolates were *Pseudomonas spp.* (6 isolates), *E.coli* (3 isolates) and *Staphylococcus spp.* (1 isolates) . Bacterial isolates were identified according to their cultural, microscopical and biochemical characteristics that were in agreement with (17).

**Table (1): The morphological ,cultural and biochemical characters of Klebsiella pneumoniae**

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indole production</td>
<td>-</td>
</tr>
<tr>
<td>Kliglar iron agar (KIA)</td>
<td>(for CO₂) +</td>
</tr>
<tr>
<td>Voges –Pproskauer</td>
<td>+</td>
</tr>
<tr>
<td>Simmons Citrate</td>
<td>+</td>
</tr>
<tr>
<td>Cell shape</td>
<td>Bacilli</td>
</tr>
<tr>
<td>MacConkey agar</td>
<td>Lactose ferment</td>
</tr>
<tr>
<td>Methyl red</td>
<td>Variable</td>
</tr>
</tbody>
</table>

**-Antibiotic Susceptibility Test**

Table (2) showed the antibiotic sensitivity screening test to a number of antibiotics on *Klebsiella pneumoniae* isolates by using Kirby-Bauer disk diffusion method .The results were interpreted according to the diameter of inhibition zones and compared with inhibition zones
determined by CLSI, and to decide the susceptibility of bacteria to antimicrobial agent whether being resistant.

The results revealed that the isolates of *Klebsiella pneumoniae* exhibited highly sensitivity to Azithromycin, moderate sensitivity for Gentamicin and Imipenem while The findings showed absolute resistant to Erythromycin, highly resistant to Ciprofloxacin, Gentamicin and Imipenem.

Erythromycin has been regarded for many years as possessing a good spectrum of activity and safety record for the treatment of respiratory, skin, and soft tissue infections in both adults and children. Azithromycin which differs from erythromycin chemically by a methyl-substituted nitrogen in the macrolide ring This difference produces improvements in spectrum and potency compared with erythromycin\(^{(18)}\).

Results showed also that the resistance of *K. pneumoniae* isolates to the aminoglycosids group included gentamicin were 50 %. \(^{(19)}\) was rather different from the data. They reported that about 79 % of *Klebsiella* isolates were resistance to gentamicin. In addition, \(^{(20)}\) found that all the *Klebsiella* strains in their studies were resistant to gentamicin. In this regard, \(^{(21)}\) found that *Klebsiella* isolates producing extended spectrum \(\beta\)-lactamase enzymes were resistant to aminoglycosids.

Resistance of *Klebsiella* isolates to ciprofloxacin which belongs to the quinolones group was 20 %, \(^{(22)}\) reported that the resistance to quinolones is related to change in antibiotic-enzyme (GyrA) binding site.

\(^{(23)}\) reported that carbapenem (imipenem) antibiotics have strong activity against ESBLs from *Klebsiella spp*. \(^{(23)}\) observed that *K. pneumoniae* isolated from respiratory tract was sensitive against imipenem. In another study *K. pneumoniae* isolated from different clinical specimens was susceptible to imipenem \(^{(24)}\). The result of the susceptibility of *K. pneumoniae* to the imipenem correlated with results of \(^{(25)}\) and \(^{(26)}\) who revealed that only one isolate was resistant to the imipenem.

While resistance to Sulfonamide group included Trimethoprim + Sulphamethoxazole) was 77.5 %, This blocks two consecutive steps in bacterial biosynthesis of essential nucleic acids and proteins and is usually bactericidal \(^{(26)}\). According to this result it can considered that Azithromycin as best treatment against *K. pneumoniae*.

**Table (2): Antibiogram for *Klebsiella pneumoniae* Isolates**

<table>
<thead>
<tr>
<th>Antimicrobial Class</th>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythromycin</td>
<td>0</td>
<td>0</td>
<td>10(100%)</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>10(100%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>5(50%)</td>
<td>0</td>
<td>5(50%)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>3(30%)</td>
<td>2(20%)</td>
<td>5(50%)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>5(50%)</td>
<td>0</td>
<td>5(50%)</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>1(10%)</td>
<td>0</td>
<td>9(90%)</td>
</tr>
</tbody>
</table>
3. Molecular Study (Genotypic Detection of *K. pneumoniae*)

A collection of 15 *Klebsiella pneumoniae* isolates diagnosed by the morphological, cultural and biochemical characters, the identification was confirmed by molecular method for the presence of *magA*. The results showed that only 10 (66.6%) isolates were carrying *magA* which are diagnosed as *Klebsiella pneumoniae*, these results were correlate with (27) who found that out of 10² *Klebsiella* isolates, 96.2% was identified as *K. pneumoniae* as in the figure (3).

**Conflict of Interest**: There was no interest in this study.

**Source of funding**: By the authors themselves.

**Ethical Clearance**: Ethical clearance was obtained from Faculty of Science, University of Kufa, Iraq.

**Acknowledgment**: The authors are thankful to Prof. Dr. Ali Abid Abojassim for their help in this work.

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of Klebsiella pneumoniae isolates from the respiratory tract.


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Immunomodulation of Iraqi Lyciumbarbarium Carotene

**in vitro**

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Abstract

The contents of Lyciumbarbarum active constituents as wild Iraqi plant was extracted then estimated qualitatively and quantitatively by HPLC method. Effect of the extracted carotene was employed in the current study on the normal human blood lymphocytes to estimate the extract effect on IL-6 and IL-8 levels as immune modulating agent. L.barbarum considered as a good source for carotenes it’s containedwas 0.287mg/g dried fruits. The current study results about the effect of extracted carotene on normal human lymphocytes and interleukins 6 and 8 levels showed that the compound can enhance cell-mediated immune responses through enhance lymphocytes proliferation with increasing in IL-8 and decrease IL-6 level.

**Keywords:** Lyciumbarbarum active, lymphocytes, L.barbarum considered, proliferation, carotenes.

**Introduction**

Wild Iraqi Lyciumbarbarumis rich with carotenoids that make the fruit possessed a beautiful orang color(1). Lycium was very important medicinal plant that possessed several biological activities, among them; hepatic protection from being damage, immune boosting agent, and can reduce the harmful effects of the chemotherapy drugs and radiotherapy treatment(2), also the regular carotene intake with daily foods as rich carotene vegetables diets might play important rule in reduction of different types of cancer(3). Due to the potent antioxidant properties of such compounds, many researches correlated between their importance in curing several several chronic disease as well as a potent biological effects on human(2). Moreover the immune modulation effects of beta carotene were suggested to be a major factor in fighting cancer, by boosting the immune system(4). This study projected on extraction of total carotene from Wild L.barbarum to insure the immune boosting activity for this components and declare how it can regulate some interleukin levels of a normal human blood lymphocytes culture.

**Material and Method**

**Extraction the total carotene from the fruit**(5): 

Ripe L.barbarumfruits were dried and powdered by blender. A quantity of one gram dried fruit powdered was homogenized well with 3ml distilled water, to be mixed then with 2ml absolute ethanol with continues agitation. After filtration, total carotene was extracted by 10 ml n-hexane using separator fennel. The organic hexane layer was objected to HPLC assay.

**Immunomodulation Determination (in vitro)**(6).

In this study about five ml blood samples was taken from peripheral vein of healthy volunteers with age of (25-35) year’s old whom never using any drugs before 2 weeks. Each blood sample was suspended onto 3ml lymphocyte separation fluid(sp.gr.1.077g/L) in vacuumed tubes separately to be centrifuged then for half an hour at2000 rpm. The isolated cell of lymphocytes were collected by sterile Pasteur pipette and transfer each sample into separated vacuumed tubes after washing the pellets, then suspended in with 5ml RPMI -1640 containing 10% fetal calf serum. All tubes were incubated over night at37°C in 5% CO₂ incubator.

**i-Measurement of the Viable Lymphocytes by MTT Assay**(7).
According to Freshney 2012 protocol, the effect of extracted carotene on normal lymphocyte culture was determined by preparing different carotene concentrations (500, 250, 125, 62.5, 31.25, 15.625, and 7.8125) µg/ml, then sterilized with disposable millipore filter. Aliquot of 100 µl lymphocyte cell culture was treated with each carotene concentrations in triplicate with control positive that employed as 0.1% PHA solution (phytohemagglutinin), and control negative represented by cells suspended in medium without any treatment. The 96 well plate was incubated for 20 hours, then centrifuged for 5 minutes at 1500 rpm. The medium was gently aspirated and MTT dye (2 mg/ml) to all wells was added. The microtiterplate was incubated again for about 3-4 hours. Crystals were formed by living lymphocytes might dissolved with 100 µl DMSO and the intensity of purple color for each well was read at 620 nm and recorded within short period by ELISA reader.

- Percentage of viable Lymphocytes can be calculated with the following equation:

\[ \text{Percentage of viable Lymphocytes} = \frac{\text{Absorbance of the test}}{\text{Absorbance of negative control}} \times 100. \]

Determination of the Cytokine Level by ELISA Technique

For this assay two tissue culture plates of 24 wells, were seeded with 1 ml of isolated lymphocytes suspended cells (1 x 10^6 cells/well). One plate was treated with 1 ml from the extracted carotene in three selected concentrations (100, 500, 250) µg/ml in triplicate to be incubated for two hours, while the other plate incubated for four hours after treatment. Lymphocyte cells in growth medium alone was represented the negative control.

All wells content were pooled at the end of exposure time the end of each interval times into sterile tubes and centrifuged for 10 minutes at 2000 rpm to separate the pellets from the supernatant of each tube and kept at -20°C to be estimated by ELISA kits for IL6 and IL-8 assy.

The Cytokine IL-6 and IL-8 Levels

The kit work was done according to protocol of United State Biological and Biochemical Reagents Trade Company specific for (IL-6 and IL-8) level, that can be calculated through plotting standard curve between concentration versus absorbance read by ELISA microplate reader (Olympus/Japan) at 450 nm were plotted, then IL-6 and IL-8 levels for each sample was calculated and then evaluated statistically.

Results

Content of Total Carotene Determination by HPLC

The retention time for the β-carotene standard was (6.647 minutes) as shown in figure (1). HPLC chromatogram of the *Lycium barbarum* extracted carotene showed a presence of sharp peak with retention time (6.638) minutes, figure (2).

![Figure 1](https://example.com/fig1.png)

**Figure (1)** HPLC Chromatogram of the standard β-carotene
Total extracted carotene concentration can be calculated through data applied for area under the curve at retention time 6.638 minutes of the extract and 6.647 minutes of the standard with the following equation:
MTT Result

Lymphocyte Proliferation determination by MTT assay:

All Results were summarized in Figure(1) showed that the seven concentrations of extracted carotene affect normal human Lymphocyte by proliferation in corresponding with negative control.

Levels of IL-6

Table (1) showed the average absorption of standard solutions for IL-6, from which a standard curve, figure (2), was plotted to get the straight line equation

<table>
<thead>
<tr>
<th>IL-6 standard concentration (pg/ml)</th>
<th>Average absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.156</td>
<td>0.144</td>
</tr>
<tr>
<td>0.312</td>
<td>0.112</td>
</tr>
<tr>
<td>0.625</td>
<td>0.117</td>
</tr>
<tr>
<td>1.25</td>
<td>0.38</td>
</tr>
<tr>
<td>2.5</td>
<td>1.093</td>
</tr>
<tr>
<td>5</td>
<td>1.411</td>
</tr>
<tr>
<td>10</td>
<td>1.978</td>
</tr>
</tbody>
</table>
Levels of IL-6 from lymphocytes treated with three concentrations of extracted carotene were shown in table(2) after two intervals 2 and 4 hours of exposure.

Interleukin 6 (IL-6) encoded by the IL6 gene is produced from different cells such as smooth cells in blood vessels. The IL-6 main action was indicated as a pro-inflammatory cytokine and myokine\(^9\), which mediated by inhibition the secretion of TNF-\(\alpha\) and IL-1 with activation of IL-10\(^{10}\). A decrease in IL-6 level secreted from treated lymphocytes with carotene in different concentration and 2 or 4 hours exposure, were shown in the current study. Dietary carotenoids and retinoids played an obvious role in boosting human’s innate and acquired immunity against inflammation\(^{11}\). The current research could be a preliminary in vitro study involve the beneficial effect of *L. barbarum* carotene, in spite that in a study concluded that the carotene decrease IL-6 level due to its suppression of transcription of this interleukin\(^{12}\).

\[ Y = 0.2044X + 0.1475 \]

\[ \{X = Y - 0.1475/0.2044 \text{ pg/ml}\} \] calculation of the IL-6 level.
Levels of IL-8:

![Standard Curve for IL-8](image)

Figure(4) Interleukin-8 standard curve

Figure(4) showed a standard curve for IL-8 and the straight line equation

\[ Y = 0.0009X + 0.1574 \]

\[ X = Y - 0.1574 / 0.0009 \] calculating IL-8 level (pg/ml)

After application of IL-8 equation, the levels of treated lymphocytes with three concentrations from the extracted carotene were determined for 2 and 4 hours intervals, table(2).

**Table(2) Level of IL-8 for Lymphocytes treated with Lycium carotene**

<table>
<thead>
<tr>
<th>Lyciumcarotene Extract concentration(mg/ml)</th>
<th>IL-8 Level(pg/ml) After 2hr.exposure</th>
<th>IL-8 Level(pg/ml) After 4hr. exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>125</td>
<td>2922.8888</td>
<td>3167.333</td>
</tr>
<tr>
<td>250</td>
<td>2951.7777</td>
<td>2856.2222</td>
</tr>
<tr>
<td>500</td>
<td>2986.8888</td>
<td>3062.8888</td>
</tr>
<tr>
<td>Control</td>
<td>2799.000</td>
<td>2799.550</td>
</tr>
</tbody>
</table>

Results showed that IL-8 level elevated for blood lymphocytes treated with different concentrations and two intervals time of exposure. Interleukin 8 secreted by macrophages and epithelial orendothelial cells, is an important mediator of the innate immune system (9). IL-8 acted as neutrophil chemotactic factor by induction of chemo-taxis for neutrophils and granulocytes, in order to migrate toward infection. Moreover, it acted to stimulate phagocytosis at site of inflammation as in bronchitis and respiratory viral diseases (13). *Lyciumbarbaum* carotene play important role in treating different diseases via improvement of body immunity(14).
The current results were agreed with a study by Lin and co about the effect of edible carotene on normal peripheral blood lymphocytes(15).

Conclusion

The contents of *Lycium barbarum* active constituents as wild Iraqi plant was considered a good source for carotene which contained 0.287mg/g dried fruits. The current study focused on the effect of extracted carotene on normal human lymphocytes and interleukin 6 and 8 levels. The study general conclusion insured that this compound can enhance cell-mediated response for immune system. *Lycium barbarum* carotene possess major role in boosting human immunity that highlighted their importance as food as well as a promise medical component.

Acknowledgment: The authors are grateful to Researchers at the Biotechnology Research Center, (University of Al-Nahrain) for their scientific support.

Conflict of Interest: There is no conflict of interest among the authors.

Funding: Self

Ethical Clearance: This study is ethically approved by the Institutional ethical Committee.

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Activity of Biosynthesized Reduced Graphene Oxide against Multidrug resistant Uropathogenic Bacteria

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Abstract

The effect of Bacterially Reduced Graphene Oxide (BRGO) as inhibitory agent alone and in combination with antibiotics against some multidrug resistant (MDR) uropathogenic bacterial isolates as well as the antibiofilm activity was investigated. BRGO nanosheets were synthesized biologically by Escherichia coli strain E-NO.7 (accession no. MK685205). Different concentrations 0.1, 0.5, 1 and 10 mg/ml of BRGO nanosheets showed potent inhibitory effects of all concentrations against tested MDR uropathogenic Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, and Methicillin-Resistant S. aureus (MRSA) isolates. Results showed that the growth inhibition zones increased with increasing in BRGO concentration. The combinations of BRGO (0.5 mg/ml) and different broad spectrum antibiotics exhibited enhanced antibacterial activity against all the studied isolates in comparison with the effects of antibiotics alone. It has been observed that BRGO effectively restricted biofilm formation, and the antibiofilm effect was dose- dependent, since the biofilm inhibition gradually increased with increasing in BRGO concentration.

Key words: Uropathogenic bacteria, Bacterially Reduced Graphene Oxide, Antibacterial activity, Antibiofilm effect.

Introduction

Urinary tract infections (UTIs) are among the most common types of bacterial infections occurring in both the community and hospital settings (1) Gram-negative bacteria, specifically Enterobacteriaceae, are common causes of both community and hospital associated UTIs. The most common pathogenic organism is uropathogenic Escherichia coli (UPEC), responsible for 80% or more of the cases, while other Gram- negative rods and Gram- positive cocci, such as Staphylococcus saprophyticus and enterococci, are responsible for the remaining cases (2).

In spite of the availability and use of the antimicrobial drugs, UTIs caused by bacteria have been showing increasing trends. Much of this increase has been related to the emergence of antibiotic resistance among urinary tract pathogens. The widespread use of broad-spectrum antibiotics has led to the appearance of MDR isolates (3). Biofilms are considered an important virulence factor that causes persistent chronic and recurrent infections. They are highly resistant to antibiotics and host immune defenses (4). Bacteria protected within biofilms are up to 1,000 fold more resistant to antibiotics than their planktonic counterparts, which generates serious consequences for therapy and complicates treatment options (5) Unfortunately, antimicrobial resistance is still a major cause of morbidity and mortality. Each year more than 700,000 people die due to antimicrobial resistant infections worldwide. Antibiotic resistance is estimated to cause around 10 million deaths by 2050 (6).

Nanomaterials (NMs) have emerged as a novel alternative to defeat MDR bacteria because of their microbicidal nature and the development of bacterial resistance to NMs are less likely when compared to antibiotics (7). The physical structure of the NM itself may have inherent antibacterial properties due to its membrane damaging abrasiveness, as seen in graphene nanosheets (8). Graphene materials have potent broad-spectrum antibacterial activities against both Gram-
positive and Gram-negative bacteria and biofilm forming microorganisms. The unique properties of graphene provide surfaces with anti-adhesive properties and it is particularly effective to inhibit bacterial attachment and the biofilm formation (9).

**Experimental part**

**Bacterial isolates**

Four uropathogenic isolates *E. coli*, *K. pneumonia*, *P. aeruginosa*, and MRSA were identified according to morphological, biochemical and VITEK2 System as MDR after screening their antibiotic sensitivity against 12 selected antimicrobials of different classes using disc diffusion method. MDR uropathogens were also identified as biofilm producers by Congo Red Agar (CRA) according to the method described by (10) and by Tissue culture plate (TCP) method according to (11, 12). The interpretation of biofilm production was done according to (13).

**Antibacterial activity of BRGO**

The antibacterial activity of BRGO nanosheets was tested against the MDR uropathogens by agar well diffusion method (14).

**Combination of antibiotics and BRGO**

The combination between BRGO nanosheets and antibiotics against uropathogens was done using the Kirby- Bauer disk diffusion method. Each standard antibiotic disc was impregnated with 30μl of BRGO solution (0.5 mg/ml) and used against indicated bacterial isolates to assay their antibacterial activity. The plates were incubated at 37°C for 24 hr. After incubation, the plates were checked for the zones of growth inhibition.

The combination effect of antibiotics and BRGO was evaluated by calculating the increase in the zones of inhibition (15).

**Antibiofilm activity of BRGO**

Tissue culture plate method (TCP) was also used with some modifications to detect the antibiofilm activity of BRGO solution against biofilm producers in vitro. Three concentrations 1, 0.5, and 0.1 mg/ml of BRGO solution were prepared. Bacterial suspensions were prepared for each isolate as, 150μl of the bacterial suspensions were added to each polystyrene 96-well microtiter plate wells. Then an amount of 50μl of each 4X concentration was added to the corresponding wells to get the final concentrations. An amount of 200μl of autoclaved distilled water was added in peripheral wells to decrease loss of water. Microtiter plates were incubated in 37°C for overnight. After incubation, contents of each well of microtiter plates were treated and the OD values were measured.

**Results**

**Antibacterial activity of BRGO**

Results showed that BRGO had potent inhibitory effects against all the tested MDR uropathogenic isolates with higher activity against MRSA. The growth inhibition zones clearly revealed that BRGO inhibited the growth of tested isolates where the highest concentration of BRGO has the strongest antibacterial activity (Figure 1, Table 1). Treatment with different concentrations of BRGO nanosheets showed higher antibacterial activity against *E. coli* compared to other Gram-negative (*K. pneumonia* and *P. aeruginosa*), the growth inhibition zones increased in a concentration dependent manner.

![Fig. 1- Antibacterial activity of different concentrations of BRGO against MDR uropathogenic isolates. C, represents control (D. W.).](image-url)
Table 1. Growth inhibition zones of different concentrations of BRGO nanosheets against MDR uropathogens.

<table>
<thead>
<tr>
<th>BRGO MDR Bacteria</th>
<th>0.1 mg/ml</th>
<th>0.5 mg/ml</th>
<th>1 mg/ml</th>
<th>10 mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Zone of inhibition (mm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>E. coli</strong></td>
<td>16</td>
<td>22</td>
<td>23</td>
<td>25</td>
</tr>
<tr>
<td><strong>K. pneumonia</strong></td>
<td>15</td>
<td>18</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td><strong>P. aeruginosa</strong></td>
<td>10</td>
<td>12</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td><strong>MRSA</strong></td>
<td>21</td>
<td>22</td>
<td>24</td>
<td>35</td>
</tr>
</tbody>
</table>

BRGO, Bacterially Reduced Graphene Oxide; MDR, Multi Drug Resistance

Combination effect of antibiotics and BRGO

The selected antibiotics were divided into two groups, the first group included four different β-lactam antibiotics (cefepime, ceftriaxone, cefazolin, and cefotaxim) that were not effective against the studied isolates, while the second group included imipenem and gentamicin that were highly active against these isolates. Results revealed that antibiotic-BRGO combinations exhibited enhanced antibacterial activities against all the studied isolates as compared to the effect of antibiotics alone with higher activity against MRSA (Table 2 Figure 2 and Figure 3).

Table 2. Individual and combined activity of antibiotics and BRGO nanosheets (0.5 mg/ml) against MDR uropathogenic isolates.

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th>E. coli</th>
<th>K. pneumonia</th>
<th>P. aeruginosa</th>
<th>MRSA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Zone of inhibition (mm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEP</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>FEP + BRGO</td>
<td>10</td>
<td>9</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>CTX</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CTX + BRGO</td>
<td>9</td>
<td>10</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>CRO</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>CRO + BRGO</td>
<td>11</td>
<td>10</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>CZ</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CZ + BRGO</td>
<td>10</td>
<td>11</td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td>IPM</td>
<td>32</td>
<td>28</td>
<td>30</td>
<td>36</td>
</tr>
<tr>
<td>IPM + BRGO</td>
<td>34</td>
<td>30</td>
<td>33</td>
<td>38</td>
</tr>
<tr>
<td>CN</td>
<td>18</td>
<td>18</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>CN + BRGO</td>
<td>25</td>
<td>20</td>
<td>18</td>
<td>24</td>
</tr>
</tbody>
</table>

FEP, Cefepime; CTX, Cefotaxime; CRO, Ceftriaxone; CZ, Cefazolin; IPM, Imipenem; CN, Gentamicin; BRGO, Bacterially Reduced Graphene Oxide.
Biofilm formation by uropathogens

Multidrug resistant uropathogens (E. coli, K. pneumoniae, P. aeruginosa, and MRSA) were identified as biofilm producers by CRA method, it was observed that all isolates produced black colonies which is indication for the production of biofilm. The experiment of TCP was performed in triplicate. The OD values of stained adherent biofilm was determined with an ELISA reader at wavelength of 590 nm. The average of OD values obtained for individual isolate were considered as an index of bacterial adherence to surface and biofilm formation. Results revealed that all the studied uropathogens were strong biofilm producers by TCP method [Table 3]. The average OD value (2.034) for P. aeruginosa indicated the strongest biofilm production, followed by 1.930, 1.575, and 1.537 for K. pneumoniae, MRSA, and E. coli, respectively.
Table 3. Antibiofilm activity of different concentrations of BRGO against MDR uropathogenic isolates.

<table>
<thead>
<tr>
<th>Bacterial isolate</th>
<th>Cont.</th>
<th>Absorbance (at 590 nm) after treatment BRGO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.1 mg/ml</td>
</tr>
<tr>
<td>E. coli</td>
<td>1.537</td>
<td>1.220</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>1.930</td>
<td>1.238</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>2.034</td>
<td>1.304</td>
</tr>
<tr>
<td>MRSA</td>
<td>1.572</td>
<td>1.215</td>
</tr>
</tbody>
</table>

**Evaluation of antibiofilm activity of BRGO**

The OD values showed that BRGO effectively restricted biofilm formation of the studied uropathogens. It has been observed that the antibiofilm effect was dose-dependent, since the biofilm inhibition gradually increased with increasing in BRGO concentration (Table 3). Three different concentrations (1, 0.5, and 0.1 mg/ml) of BRGO nanosheets exhibited higher antibiofilm effect against MRSA than Gram-negative isolates. The effect of concentrations corresponding to 0.5mg/ml and 1mg/ml of BRGO caused notable reduction of the biofilm formation of MRSA from approximately 1.572 to levels of 0.619 and 0.124, respectively. The OD values also revealed that concentrations of BRGO caused notable reduction in the biofilm formation of E. coli and K. pneumoniae and the best biofilm inhibition activity was observed in higher concentration. The effect of concentrations corresponding to 1, 0.5 and 1mg/ml of BRGO caused reduction of the biofilm formation of E. coli from approximately 1.537 to levels of 1.220, 0.620 and 0.153, respectively. Similarly, these concentrations showed reduction in biofilm formation of K. pneumoniae from approximately 1.930 levels of 1.238, 0.621, and 0.191, respectively. Against P. aeruginosa, treatment with BRGO showed relatively lower antibiofilm effects compared to the other isolates. The biofilm formation reduced from 2.034 to levels of 1.304, 0.720, and 0.215 corresponding to 1, 0.5 and 1mg/ml concentrations of BRGO respectively.

**Discussion**

Graphene nanosheets can cover on the external surface of cells, which might lead to indirect toxicity by biologically isolating them from growth medium, and consequently the bacterial cells can neither proliferate nor consume the nutrients (16). In addition, the direct contact between the sharp edges of RGO nanosheets with cells can physically damage cell membrane, resulting in leakage of intracellular material and negatively affecting cell metabolism (17). Many researchers have investigated the potential toxicity of GNMs against several bacterial species (18, 19). The increase in graphene concentration led to a continuous increase in its antibacterial activity (20). The antimicrobial effectiveness of biosynthesized RGO is enhanced at higher concentrations due to the rupture of cell membrane (21).

The inhibition ability of antibiotics combined with BRGO may be attributed to “carrier effect” (22). Namely, the coating of BRGO on bacteria can cause the cell membrane damage and will facilitate the release of the deposited antibiotic on BRGO surface in high quantity. The cell entrapment property of graphene ensures high local concentrations of antibiotic molecules in the immediate proximity of the cell membrane. It is also possible that RGO contributed to increased permeation of antibiotic into the bacterial cells (23).

Bacterial biofilms play an important role in UTIs, being responsible for both acute and persistent infections in which biofilm can become a serious problem (24). Many
studies documented a higher proportion of antibiotic resistance in biofilm producers in comparison to non-producers (25, 26). The EPS matrix confers antibiotic resistance through expression of chromosomally encoded resistant genes, restricted penetration of antibiotics, and decreased growth rate (27, 28). On the other hand, it is possible that the biofilm can facilitate the accumulation of antibiotic degrading enzymes such as β-lactamases (29).

The penetration and deposition of NMs within the biofilms are key components for the design of biofilm therapeutics. The bioavailability of NMs allows them to penetrate a mature biofilm and target bacterial cells not only at the surface but also within the deep layers of biofilm (30). The antimicrobial and antibiofilm effect of graphene can be attributed to different mechanisms originated by the direct interaction between graphene and bacteria cells (31). Graphene nanosheets possess biocidal properties based on their ability to act as nano-knives, penetrating and disrupting the cell membrane (32). Additionally, their basal planes which are free of functional groups possesses anti-adhesion properties against biofilm and it is particularly effective in preventing biofilm formation (9).

The BRGO nanosheets exhibited remarkable antibacterial and antibiofilm activities against the tested MDR uropathogenic E. coli, K. pneumonia, P. aeruginosa, and MRSA isolates. The activity increased with increasing in BRGO concentration. The combination of BRGO and broad-spectrum antibiotics showed enhanced antibacterial activity against uropathogens as compared to effects of antibiotics alone.

Conflict of Interest: Nil.

Source of Funding: Authors have no competing interests.

Ethical Clearance: Authors are in accordance with the ethical standards of the responsible committee on human experimentation (institution and national) and the Helsinki Declaration of 1975.

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Legal Protection Against Women in Sexual Violence

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Abstract

Introduction: Sexual violence is one of the morbidities and mortality factors in women. Sexual violence of women causes physical and physiological sequences that make them vulnerable to health problems.

Material and method: It was normative legal research with a literature study and document study as a method to collect the research data.

Findings: Marital sexual abuse or the highest personal relation was incest with 1,071 cases followed by raping with 818 cases, and molestation with 321 cases. Meanwhile, marital rape reached 195 cases. The forms of sexual violence consist of sexual harassment, sexual exploitation, coercion of contraceptive use, compulsion in abortion, rape, marital compulsion, prostitution, sexual bondage, and sexual torture. The protection against victims of sexual violence was considered to be not optimal due to a lack of public understanding of the causes and effects of sexual violence.

Conclusion: So far, the legal protection against women in sexual violence cases have not been optimally implemented. The minimum legal protection triggered impunity, reoccurrence, and even the victims’ frustration to accept justice and recovery.

Keywords: Legal Protection, Sexual Violence, Women

Introduction

Sexual violence is considered a serious human right violation with a huge impact, especially for women1. Sexual violence is also a form of violence frequently experienced by women related to the stereotype that put women as an object of sexuality2. It can be stated as violence against women, gender-based violence, and gender violence3. Any form of sexual violence can be categorized as violence against morality and domestic violence. This act is characterized by some indicators such as carried out in the house, behind the closed door, involves physical violence, performed by people with a close relationship to the victim, and reoccurring. The victims are usually women and children, with men (father or husband) mostly as the subject of the act. However, in some kinds of literature, domestic violence refers to violence performed by a husband to his wife or also known as wife beating4.

In Indonesia, sexual violence is divided into three categories, which are molestation (911 cases), sexual harassment (704 cases), and rape (699 cases). The high rate of sexual harassment leads to weak legal protection against women5. It is more difficult to reveal and handle sexual violence compared to the other forms of violence as it is frequently correlated to society’s concept of morality. Women are considered as the symbol of purity and dignity. Therefore, it is such a shame to be the object of sexual violence. It makes women tend to be silent when they are involved in sexual violence6. Sexual violence is the main factor of morbidities and mortality in women. Sexual violence of women causes physical and physiological sequences that make them vulnerable to health problem7. Women face significant risks such as genital and extragenital trauma, an unexpected pregnancy, sexual dysfunction, and sexually transmitted disease (STD)8.

The draft of the law about sexual violence is aimed to prevent sexual violence, act against the subject of sexual violence (includes rehabilitation), victim’s recovery, implement the duty of a country to remove violence against women9. Victim protection during the judicial process strongly relates to the applied legal provisions. Today, the law about sexual violence already exists,
but it has not been optimally implemented. The scope of sexual violence is mentioned in Law No. 23 of 2004 about the elimination of domestic violence and Law No. 39 of 1999 about human rights mentions the limited scope of criminal justice for sexual violence which limits the criminal act and forms of sexual violence experienced by victims. According to the law, the victim of sexual violence can only get protection by following the legal process. Therefore, legal protection is needed for women as victims of sexual violence.

Method

It was normative legal research, which is research about legal principles. The research focused on the material of primary and secondary law. It was descriptive research that gave a detailed and systematic description of legal protection against women in sexual violence. The data was collected through literature and document study. The literature study was done by analyzing the law and regulations, books, literature, and other research papers. The research data was qualitatively, completely, and comprehensively analyzed to produce empiric-normative legal research. The data collected from the research was analyzed and systematically outlined to conclude.

Results and Discussion

The sexual violence mostly experienced by victims within their family in a form of domestic violence or their private are. The form of domestic violence was dominated by incest (1,071 cases), followed by rape (818 cases) and molestation with 321 cases. In 2017, a surprising finding mentioned 1,210 cases of incest. Meanwhile, in this year, the incest case was reduced to 1,071 cases. The level of marital rape was quite high with 195 cases; meanwhile, marital rape in 2017 was 172 cases. It meant that the marital rape report was increased from 172 to 195 reports and needed to be taken seriously. The courage to report the marital rape cases showed that the victims were more aware of the coercion of sexual intercourse was considered as marital rape which can be sued.

There was no comprehensive protection mechanism as the scope of the cases was wider than the related regulation. The impact of the minimum legal protection triggered impunity, reoccurrence, and even victims’ frustration of justice and recovery. On the other hand, the existed law and judgment were not able to prevent and handle the case using due diligence principles. Here are the forms of sexual violence reported based on the victims’ experience and complaint.

1. Sexual harassment

Sexual harassment was not identified in the Criminal Code of Indonesia; the section of morality violence only mentioned adultery, molestation, and rape which the criminal act was in the form of physical activity. On the other hand, in the real situation, sexual harassment may in a form of non-physical contact such as verbal insult and attack that made someone feel humiliated and insulted. The draft of the law about sexual harassment mentioned sexual harassment involves physical and non-physical harassment. Some examples of sexual harassment cases were: A non-physical and insulting sexual harassment experienced by BN, an administration employee in a state school in Mataram, NTB. It was said that the school principals said about his sexual fantasy of BN and pursued BN to fulfill his sexual need either on the phone or when they went face to face. It insulted BN and made her uncomfortable. Meanwhile, the local cop of NTB considered the criminal act as molestation as there was no physical contact. Besides, he also showed some sexual content either from social media or from his document to the victim.

Physical sexual harassment in affirmative law is also known as molestation, of which the definition ranges very wide as it also includes sexual intercourse. Meanwhile, the mentioned sexual harassment above didn’t include sexual intercourse and categorized as a morality violation. The National Committee on Violence against Women (Komnas Perempuan) found many forms of physical sexual harassment. The physical sexual harassment frequently aimed women’s breasts as the target in some situations including the act done by a medical practitioner or employees in any asylum or social orphanage when they cleaned their patients’ bodies in an open space in which people can see the patients easily. The employees thought that the patients would feel ashamed of their bodies. Besides, in 2018 a physical sexual harassment was experienced by a commuter line train passenger. A male passenger rubbed his sexual organ on her body. She tried to move, but the man did the same thing again. However, despite the shock and anger, the female passenger could not do anything. She then felt so much regret to be angry but could not fight against it.
2. **Sexual exploitation**

Sexual exploitation is attached in Law No. 21 of 2007 about human trafficking eradication as the objectives, and also in Law No 35 of 2014 as the revision of Law No 23 of 2002 about protection for children. Sexual exploitation includes a sexual activity that tends to take benefit from the object and mainly for the subject’s benefit only. Sexual exploitation had not been included in the Criminal Code and Law as a form of sexual violence. Therefore, any cases related to sexual exploitation rarely forwarded into the legal court. The modus of this case usually happened when women were promised to work abroad with a high salary, but they ended up being trafficked or raped by a foreigner. As an example, SF was promised to work abroad. Although she canceled her intention after experiencing sexual violation, the agent asked her to pay a huge amount of money as the substitution of administration fee the agency was spent for her during the training. SF forced to do the sexual activity for the agency benefit, or she would be fined. It was a clear form of sexual exploitation.

3. **Rape**

Rape commonly involves coercion, manipulation, violence, and threat. Sometimes, sexual intercourse does not only involve sexual organ penetration but other things besides the sexual organs such as finger or other kinds of stuff. The act also includes sexual activity with children, also take benefits from the powerlessness, inability, and disability of the victim. Those activities are mentioned as rape in the bill of sexual violence elimination. The National Committee of Violence Against Women identified a rape case back in 2018 which was experienced by AG, a college student of UGM in her internship from June – August 2017 in Maluku. Her fellow member, HS raped AG in her sleep. AG went through a long way to get justice. However, the compensation was not equal to what she expected. Instead, the university tended to blame AG with a statement that cornered AG as a woman who pursued the man to get into the sexual activity, of which no man would not deny accepting that. The case was then closed on February 4th, 2019 through the non-litigation process. No legal process was applied for this case. Both HS and AG were allowed to finish their studies and gave them counseling. HS was asked to apologize in front of the UGM commissionaires and HS parents. Meanwhile, AG was compensated with a scholarship until she finished her degree. the other case was experienced by NA, a woman who was raped by her neighbor named Marhon.

4. **Sexual bondage**

Sexual bondage is a form of sexual violence which is aimed to give sexual service under the control of the subject in a certain period until the victim got helped. An example of sexual bondage found by National Committee on Violence against Women was in 2018 which was experienced by CT (27 years old). She was the only victim who was brave enough to report the sexual bondage done by Gatot Brajamusti, ex-chief of PARFI, a spiritual guru, and a leader of a hermitage. The case attracted public attention as the subject was a public figure added with the assumption that there were many women that might be involved in that case (although most of them were not brave enough to report the case) and most of the victims were very young women which were promised to be an actress or singer.

5. **Sexual torture**

Sexual torture is a form of sexual violence that was not regulated in any law and Criminal Codes. The bill of law about sexual violence elimination tries to include sexual torture in the law as a form of effort by anti-torture convention to comprehensively handle sexual torture. Sexual torture involves some acts to intentionally attack sexual organs for certain reasons so that it can cause terrible physical, spiritual, and sexual pain. A Malaysian citizen reported that she was a victim of sexual torture during the body checking in Adisucipto Airport Yogyakarta. ML arrived at Adisucipto Airport on December 31st, 2017 at 10.30. just like the other passenger, she went through the x-ray scanning, dog screening, and document checking. However, her baggage was arrested, and she was required to went for another checking. She was placed in a special room. In that room, the officers checked her belonging but did not find any forbidden stuff. However, the officers kept on asking about a package of food and her private stuff. Instead of being released, she was asked to enter another room for body checking. In that room, a female officer asked her to take off all of her clothes without any further explanation. When she was naked, she was asked to stand in front of a big mirror. She didn’t know if that mirror was only a common mirror or a two-way mirror. At that time ML was not able to do anything other than following the officers’ instruction as she did not want to be claimed as a rebel foreigner. In the end, there was no further explanation about all the security checking, and
she left with a stressed, ashamed, and humiliated feeling. It was categorized as sexual torture as it was done by those who owned the authority to do all the checking which was aimed to satisfy their assumption that ML carried drugs17.

Based on the data about sexual violence cases, the protection for women as the victims of sexual violence was considered to be not optimal due to the lack of understanding of the cause and effect of sexual violence. Besides, the legal institution in Indonesia was not able to properly protect the victims due to the limitation of regulating law18. In the affirmative law about sexual violence did not comprehensively understand the cases. The limited scope of sexual violence criminal act limited the form of sexual violence experienced by victims19. For example, sexual harassment, sexual exploitation, coercion of contraceptive use, compulsion in abortion, rape, marital compulsion, prostitution, sexual bondage, and sexual torture did not clearly state in the law. Legal protection only protected those who were brave enough to bring the case to the court20.

Sexual violence also affected both the victim and her family as they suffered from the threat, restriction, exclusion, also social, political, and economic discrimination. Further example of the impact was when the victim was blamed by the society and family, denied by the family, expelled and excluded by her society, workplace or school, forced to marry the perpetrator, got some difficulty to access citizenship documents. They also did not get any social security, economically dependent on family and close relatives, lost her beneficiary, did not get any medical and psychological treatment by considering the victim as the wrongdoer21.

To give optimum service for sexual violence victims, collaboration and coordination are needed to evenly distribute the jobs of each related institution, including the agreed mechanism, policy support, and the availability of the resource. A country should have protected its citizens from the fear, as free from fear is a basic human right.

**Conclusion**

Sexual violence case on women was considered very high and psychological mental trauma, fear, shame, anxiety, and even a suicide trial. The legal protection of women has not been well implemented. The protection mechanism was not comprehensively available due to the case complexity that surpassed the availability of the cases in the established regulation or law. The minimum legal protection triggered impunity, reoccurrence, and frustration of the victims to get justice and recovery.

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

**Ethical Clearance:** Not required

**Source of Funding:** Self-funding research

**References**


The Clinical Function Comparison of Post Operative Nerve Grafting and Nerve Transfer in Patients with Brachial Plexus Injury

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Abstract

Background: Brachial plexus injury is most commonly caused by motorcycle accidents and leads to a deficit of motor strength, pain and disability of the upper limb. The main purpose of reconstructive action of brachial plexus injury is to restore the function of the shoulder and elbow. Aim: This study aimed to compare the clinical function of post-operative nerve grafting with nerve transfer in patients with brachial plexus injury.

Methods: This study enrolled a sample of patients who had surgery and evaluated the range of active movement of shoulder abduction and elbow flexion; motor strength using the scale of the British Medical Research Council (BMRC); neuropathic pain using Visual Analog Scale (VAS); and upper extremity disability using the Quick Disability of the Arm, Shoulder, and Hand (Quick DASH) questionnaire.

Results: 30 patients were divided into groups of nerve grafting and nerve transfer with each group consisting of 15 patients. 13 patients (87%) of each group had motor strength of functional shoulder abduction (>M3) (p = 0.874). 13 patients (87%) had better functional elbow flexion motor strength (>M3) in the nerve transfer group than 11 patients (73%) of the nerve grafting group (p = 0.036).

Conclusion: The postoperative nerve transfered clinical function showed a better elbow flexion function with a lower severity of upper limb disability.

Keywords: brachial plexus injury, nerve grafting, nerve transfer.

Introduction

Brachial plexus injury is a peripheral nerve injury in the upper limb which is often diagnosed and neglected by medical practitioners because of waiting for a functional recovery, resulting in motor and sensory deficits, accompanied by pain and limitation of limb function which are mutually debilitating. Consequently, it results in a decreased life quality of patients and more negative impacts in terms of psycho-socio-economic.

As the number of survivors increases from high-speed motorcycle cidents, the number of brachial plexus injuries also increases. From several epidemiological studies in the United States and Europe, 10-20% of peripheral nerve injuries are brachial plexus injuries with a prevalence of 1.2%, in which 80-90% of such injuries are caused by motor vehicle accidents. Brachial plexus injury is found in the 15-25 year-old male patients.

The management of brachial plexus injury is still considered a futile attempt by some peripheral neurologists until the 1960s. However, it changes drastically along with the development of physiology...
knowledge and peripheral nerve reconstruction techniques. The nerve grafting technique is quite effective in bridging the gap that is too wide after the excision of the nerve segment of the brachial plexus that is damaged and contains fibrotic tissue, compared to the previous action of shortening the collarbone to perform end-to-end coaptation of the neural stump proximal to distal.

Nerve graft acts as a source of the endoneurial tube in which the axonal regeneration occurs. Autogenous nerve graft has another advantage, which is capable of providing a viable source of Schwann cells. Neurotization techniques are published in the form of direct connection of functional donor nerves to the injured recipient nerves. It has provided a major advance in the technique of brachial plexus injury neural reconstruction.

The main purpose of neural reconstruction in brachial plexus injury is to restore clinical function and achieve optimal patients’ quality of life. The success of nerve reconstruction with micro surgical techniques should be followed by the assessment of patients’ clinical function as postoperative follow-up both oriented to surgeons such as range of movement (ROM) measurement of joint activation and motor strength recovery, as well as patient-oriented assessment such as neuropathic pain severity and upper limb dysfunction. From that statement, the aim of this study to compare the clinical function of post-operative nerve grafting with nerve transfer in patients with brachial plexus injury.

Method

This study used an observational analytic study, with a retrospective cohort design, in which the researchers performed a comparative analysis of current postoperative clinical function in two groups of patients who had performed different surgical actions in the past.

The sample of the study was patients with brachial plexus injury post-operative nerve grafting and nerve transfer, and conducted on January 1, 2009 until September 30, 2014 in Dr. Soetomo Teaching Hospital Surabaya. The sampling technique applied consecutive sampling, in which each patient who fulfilled the inclusion and exclusion criteria was included in the study sample until the required sample number was obtained.

The inclusion criteria were nerve grafting and nerve transfer performed by one operator, the surgery was conducted minimal and maximum within 9 months after the trauma event, and the clinical function evaluation was conducted at least 6 months after the surgery. On the other hand, the exclusion criteria included brachial plexus injury patients who have performed reconstructive actions other than nerve grafting and nerve transfer (eg. tendon transfer, free functioning muscle transfer, arthrodesis), obstetrical brachial plexus injury and patients who were not willing to participate in the research. The procedure of data collection started from collecting medical record database of inpatient and medical record of Orthopedics and Traumatology Outpatient Dr. Soetomo Teaching Hospital Surabaya to record the population of patients with brachial plexus injury that have been encountered nerve reconstruction. The sample was grouped into two groups, post-operative nerve grafting group and nerve transfer group. Further measurements of postoperative clinical function were conducted by using Data Collection Sheet and QuickDASH questionnaire, either by meeting patients at Orthopedics and Traumatology Outpatient Dr. Soetomo Teaching Hospital or conducted home-visite.

The demographic distribution of sex, age, diagnosis, incidence time to operation (in months), and time difference of surgery until evaluation (in months) was presented using descriptive statistics of mean value, standard deviation, minimum value, maximum value, median or the value of a number in percentage. On the other hand, the normality data test was conducted by using Kolmogorov-Smirnov test. If the data was normally distributed, Independent t-test was applied. However, if the distribution of data was not normal, it assessed by using Mann-Whitney test. The results were statistically significant if $p < 0.05$. The computer program used to perform statistical analysis was SPSS program version 16.0 (SPSS Inc., Chicago, Illinois, USA).

Result

This study used a sample of 15 patients in the nerve grafting group and 15 patients in the nerve transfer group. A total 15 patients (50%) with brachial plexus injury were aged 21-30 years old (7 patients in postoperative nerve grafting group and 8 patients in postoperative nerve transfer group). On the other hand, the second most age range was 11-20 years old of 6 patients (4 patients in postoperative nerve grafting group and 2 patients in postoperative nerve transfer group). The mean age of nerve grafting group was 27.2 years old and the nerve transfer group was 29.93 years old. The
independent t-test was performed because of the normal data distribution with $p = 0.510$.

In sex distribution, brachial plexus patients were dominated by male patients as many as 24 patients (11 in post-operative nerve grafting group and 13 patients in postoperative nerve transfer group). Female patients were 6 patients (4 patients in post-operative nerve grafting group and 2 patients in postoperative nerve transfer group). In nerve grafting group, there were 11 male patients (73.3%) and 4 female patients (26.7%). The nerve transfer group obtained 13 male patients (86.7%) and 2 female patients (13.3%). The gender variable was tested with Fisher’s exact test with $p = 0.651$. There was no significant difference in gender variables.

The diagnosis of patients with major brachial plexus injury was found complete postganglionic type (C5-6-7), preganglionic type (C8-T1) of 15 patients in postoperative nerve grafting group. The rest were postoperative nerve transfer patients: 9 patients with incomplete upper injury type (C5-6) and 6 patients with incomplete upper injury type (C5-6-7). From 15 patients in nerve grafting group, the patients were complete injury type (100%). The result of Chi-square test was conducted with $p = 0.000$. There was a significant difference. The result of statistical test using independent t-test (normal data distribution) showed that there was no significant difference between the two groups, either from the time incidence until the surgery ($p = 0.052$), and from time difference of surgery until the evaluation ($p = 0.959$). Overall, the sample characteristics between the nerve grafting and nerve transfer group were homogeneous.

The result of ROM comparison of shoulder abduction showed no significant difference between nerve grafting group ($71.33° ± 32.87°$) and nerve transfer group ($61.67° ± 34.52°$). The statistical test was conducted by using independent t-test normal with $p = 0.439$. However, in contrast to elbow flexion ROM results, the nerve transfer group ($102.67° ± 36.35°$) was better than nerve grafting ($86.67° ± 48.32°$) (Table 1).

### Table 1. Comparison of Post-operative Clinical Functions

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nerve Grafting (n = 15)</th>
<th>Nerve Transfer (n = 15)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoulder abduction ROM*</td>
<td>71.330 ± 32.870</td>
<td>61.670 ± 34.520</td>
<td>0.439</td>
</tr>
<tr>
<td>Elbow flexion ROM*</td>
<td>86.670 ± 48.320</td>
<td>102.670 ± 36.350</td>
<td>0.031</td>
</tr>
<tr>
<td>Shoulder Abduction Motor (BMRC)**</td>
<td>3(2-4)</td>
<td>3(2-5)</td>
<td>0.0874</td>
</tr>
<tr>
<td>Functional Motor M &gt;3***</td>
<td>13 (87%)</td>
<td>13 (87%)</td>
<td></td>
</tr>
<tr>
<td>Elbow Flexion Motor (BMRC)**</td>
<td>3 (1-4)</td>
<td>4 (1-5)</td>
<td>0.036</td>
</tr>
<tr>
<td>Functional motor M &gt; 3***</td>
<td>11 (73%)</td>
<td>13 (87%)</td>
<td></td>
</tr>
<tr>
<td>Neuropathic Pain (VAS)**</td>
<td>2 (0-7)</td>
<td>1 (0-3)</td>
<td>0.236</td>
</tr>
<tr>
<td>Upper extremity disability (QuickDASH score)*</td>
<td>42.67 ± 21.92</td>
<td>24.60 ± 14.83</td>
<td>0.014</td>
</tr>
</tbody>
</table>
From that table the difference was statistically significant, since the independent t-test result had $p = 0.031$. The minimum motor strength of shoulder abduction on the motor scale of BMRC in the nerve grafting group was $M_2$, the maximum value was $M_4$, and the median value was $M_3$. On the other hand, the nerve transfer group obtained the minimum value of $M_2$, the maximum value of $M_5$, and the median value of $M_3$. To compare the motor strength of shoulder abduction, statistical test was assessed with Mann-Whitney test because the normality test (Kolmogorov-Smirnov test) obtained abnormal data distribution. The results obtained $p = 0.874$, where there was no significant difference between the nerve grafting group and the nerve transfer group. It corresponds to the number of samples with the motor strength of functional shoulder abduction with a value of $>M_3$ (useful motor recovery). Between the nerve grafting group and the nerve transfer was the same, i.e. 13 patients each (87%) (on table 1).

The comparison of elbow flexion motor strength showed that nerve transfer group was better than nerve grafting group. The difference was statistically significant since the results of statistical tests with Mann-Whitney test (abnormal data distribution) had $p = 0.036$. This is consistent with the number of samples with functional elbow motor strength with a value of $>M_3$ (useful motor recovery) in the nerve transfer group of 13 patients (87%), more than the nerve grafting group of 11 patients (73%) (Table 2). Based on the VAS scale in the nerve grafting group, the minimum value was 0, the maximum value was 7 and the median value was 2 (Table 2). The statistical test used was Mann-Whitney test because the distribution of data was not normal with $p = 0.236$. There was no significant difference between nerve grafting with nerve transfer group. This is in accordance with previous studies. Meanwhile, other researchers have published a recovery of motor strength $>M_3$ in 80% (total 577 patients) and 73.7% (total 19 patients) postoperative nerve transfer. The significant difference showed that nerve transfer group was better than nerve grafting. It corresponds to the number of samples with a functional elbow flexion motor strength with a value of $>M_3$ (useful motor recovery) in the nerve transfer group, more than in the nerve grafting group.

Brachial plexus injury often occurs as a result of trauma resulting in a paralysis of clinical function in the upper limb. Almost 50 years ago, there have been many reconstructive actions in patients with brachial plexus injury. The main purpose of reconstructive action on brachial plexus injury is to restore clinical function and achieve optimal patients’ quality of life.

The success of nerve reconstruction with micro surgical techniques should be followed by assessment of patients’ clinical function as postoperative follow-up (surgeon-oriented such as ROM measurements of joint active and motor strength recovery, or patient-oriented such as the severity assessment of neuropathic pain and upper limb dysfunction). In this study, there was no significant difference in shoulder abduction ROM between the nerve grafting group and nerve transfer group. The results of this study are still better than previous studies, which only obtained shoulder abduction ROM of 57$^\circ$12. Previous studies had better shoulder abduction ROM of postoperative nerve grafting results than this study of 120$^\circ$13. However, the better shoulder abduction ROM of postoperative nerve transfer result was obtained in previous studies14.

This result was also supported the operation of elbow flexion function reconstruction. It shows that all samples of nerve grafting group use sural nerve as nerve graft interposition, whereas nerve transfer group used double-fascicular nerve transfer technique published by Mackinnon. Motor power postoperative nerve transfer may be better than nerve grafting. It may be due to the occurrence of nerve re-innervation to target muscles to
The percentage of patients with functional elbow flexion motor restoration with value >M3 postoperative nerve grafting in this study was not much different from previous research results. While in patients with post-operative nerve transfer, the results of this study is also not much different from the results of research conducted by previous researchers. There was no significant difference between the nerve grafting group and the nerve transfer. However, in the nerve transfer group, it has a larger number of samples at a lower VAS scale than the nerve grafting group.

The results are consistent with previous studies that demonstrate the presence of axons undergoing regeneration via cable nerve graft, resulting in neuropathic pain postoperative nerve grafting. Other results indicate if the mean VAS value decreases postoperative nerve transfer. Then, the severity of upper limb disability based on the QuickDASH score in the nerve transfer group is lower than that of nerve grafting, resulting in significant differences. It is similar with the results of previous studies showing a lower mean score post operative nerve transfer.

**Conclusion**

Based on the result of the research, it can be concluded that elbow flexion ROM post-operative nerve transfer is greater than nerve grafting, motor elbow flexion strength post operative nerve transfer is better than nerve grafting and the upper limb disability severity postoperative nerve transfer is lower than nerve grafting.

**Conflict of Interest:** There is no conflict of interests.

**Source of Funding:** This study is self-funded.

**Ethical Clearance:** This study was approved by Ethical Commission of Health Research Faculty of Medicine University of Airlangga.

**References**


Combination Use of Aloe Vera and VEGF Promotes Osseointegration and Stability of Titanium Implants

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Abstract

Background: Aloe vera was used to enhance defense mechanisms, and it has a variety of components that affected on periodontal healing and other oral condition. It was used also around dental implants to control inflammation from bacteria contamination. Vascular endothelial growth factor (VEGF) is of particular interest because of its ability to induce neovascularization (angiogenesis), VEGF also acts to recruit and activate osteoclasts as well as stimulate osteoblast chemotaxis, differentiation, and matrix mineralization.

Aim of study: The objective of this research was to illustrate the biological actions of topical application of aloe vera and growth factor VEGF on implant tissue contact surface which is involved in osseointegration.

Materials and Method: Commercially pure Titanium (cpTi) implants with aloe vera and VEGF, were placed in the tibia of (24) New Zealand white rabbits, Mechanical test (torque removal test) was performed as an indicator for the presence of osseointegration and bone implant contact (BIC) measurement as a test for the property of bone-implant interface. Histological investigation was performed on all the implants of both control and experimental groups at (1, 2, and 6 weeks) healing intervals.

Results: Findings illustrated that removal torque mean and BIC values in experimental implant showed high value in comparison to control. Histological results show an early bone deposition and early maturation in combination group.

Conclusions: Bone formation & maturation was accelerated by adding biological materials and using a combination of Aloe Vera and VEGF is a powerful tool for enhancement of osseointegration.

Keywords: Aloe Vera, Titanium implant, Vascular endothelial growth factor, Osseointegration, Implant surface, Bone

Introduction

Osseointegration refers to a structural and functional fusion of the implant surface with the surrounding bone tissue [1,2]. Many factors may include and affect osseointegration [3] such as surgical technique [4], healthfulness of host bed [5], implant design [6], implant surface and its modification [7], material biocompatibility and loading conditions [8,9].

Aloe Vera is really quite an incredible plant, has used therapeutically for over 5000 years. [10]. The bulk of the Aloe Vera leaf is filled with a clear gel-like substance, which is approximately 99% water. Aloe Vera contains over 200 active components including vitamins, minerals, amino acids, enzymes, polysaccharide, and fatty acids. The vitamins including A, C, E, folic acid, choline, B1, B2, B3 (niacin), B6 [11]. It is also one of the few plants that contains vitamin B12, while the minerals include: calcium, magnesium, zinc, chromium, selenium, sodium,
iron, potassium, copper, manganese, and important fatty acids include HCL cholesterol (which lowers fats in the blood), campesterol, and B-sitosterol\cite{12}.

Aloe vera has been used as a popular herbal medicine since ancient times for many conditions including burns, wound healing, and in dental treatment it illustrates bone, cementum and periodontal ligament regeneration\cite{13,14}.

Vascular endothelial growth factor (VEGF) is endothelial cell-specific mitogen that suggested a regulator of naturally occurring physiologic and pathologic angiogenesis. It stimulates endothelial cell growth, angiogenesis, and capillary permeability that accelerate healing events\cite{15}.

The present study was planned to use an external local application of Aloe Vera and VEGF with implantation of screw and study their effect on healing and osseointegration process.

**Materials and Method**

**Animals**

A total of 24 male New Zealand White rabbits, weighing (2.5-3 kg), aged (10-12 months) were used in this study, and kept in the animal department of (National Center of Drug Control and Research /Iraq) at a constant humidity and temperature of 23°C according to the National Council’s guide for the care of laboratory animals.

**Materials**

- Commercially pure titanium (CpTi) rods
- Aloe vera Gel 87.399%, Phyto care company.
- VEGF165, human recombinant, Bio Vision company

**Methods**

Commercially pure titanium (CpTi) rods (30 cm in length and 5 mm in diameter) were used to form (96) screw-shaped implants by using lathe machine. Each screw was 8 mm in length (5 mm threaded and 3 mm smooth) and the diameter was 3.5 mm in the threaded part and 4mm in the smooth part. The bottom of the screw was made flat but the head of the screw had a slit with 1.5 mm in depth to fit the screw driver of the torque meter during insertion and removal of the implant. Then the implants were cleaned by using ultrasonic cleaning bath (Sonomatic/170-2-T80, Germany) with ethanol and acetone of 75:25 wt% ratio was carried out to removing debris and contamination from the fabricated samples. Implants were rinsed with distilled water and then dried in the oven at temperature of 100 °C. Then the implants were sterilized by using gamma radiation with a CO\textsuperscript{60} source.

Ninety six Implants were divided as follows:

- Group I : Control : 24 Ti implant inserted in rabbit tibia
- Group II: Experimental consists 3 subgroups
  - A: 24 implant inserted in rabbit tibia with local application of 0.1µl of VEGF
  - B: 24 implant inserted in rabbit tibia with local application of 0.1µl of Aloe Vera
  - C: 24 implant inserted in rabbit tibia with local application of combination of 0.1µl VEGF and 0.1µl Aloe Vera

**Surgical procedure:**

Animals were anaesthetized generally with a mixture of ketamine (50 mg/kg) with xylazine(2.5 mg/kg). Surgical technique was performed to prepare holes, two holes in the left tibia in which control was inserted in one hole, & experimental (VEGF) was placed in the second one with a space 2 cm. In the right tibia one hole was prepared for the insertion of implant with experimental (AV), and other one for combination of (VEGF and AV) with a space 2 cm.

Each hole was washed by normal saline and dried gently by air. For the experimental group; 0.1µl of VEGF was applied in socket hole by using a micro-pipette and was left for one minute, while hole with 0.1µl of AV was left for two minute. Application of a combination was done by using VEGF firstly, allow one minute followed by adding Aloe Vera and give two minutes, then insert the implant.

Eighteen animals were sacrificed after 1, 2, and 8 weeks (6 animals for each period) for histological examination.

Bone implant contact (BIC), as histomorphometry at the light microscopic level was done for the period of 8th week.
The rest sixth animals were subjected to mechanical test (torque removal test). The torque was measured with the torque wrench device (OstitelTM; Savedalen, Sweden)[16].

**Radiographic evaluation**

X-ray was done prior to surgery, to ensure that the bone of the tibia was sufficient to accept the implants, and immediately after the operation to ensure that the implants were properly inserted in their positions. After each healing period, both tibiae were examined radiographically again to assess bone healing around each implant.

**Specimen retrieval**

The implants along with their surrounding bone were excised with a surgical saw right away following the euthanasia. The excess tissue was dissected and the specimens were removed with a margin of surrounding bone of about 5–10 mm. The specimens were immediately put into the 10% formaldehyde solution.

**Sample preparation for embedding**

The specimens were decalcified, washed and then dehydrated in the ascending graded ethanol solution. Specimens were embedded in wax block and trimmed, the first undefined slice was removed from the saw blade and the desired section thickness was selected. Mounting and staining of slides by hematoxylin and Eosin (H&E).

**Statistical methods**

Mean values and standard deviations were calculated for Removal Torque test and bone implant contact (BIC) in different studied groups at sixth week measured period. ANOVA test was used to analyze the differences between the implant groups. P value <0.05 was considered significant.

**Results**

**Radiographical evaluation** The results appeared that there were no gross changes in the tibial architecture with no areas of radiolucency between implant and adjacent cortical bone in all specimens, figure(1)

Figure(1) Conventional radiographic view for implant in rabbit tibia

**Mechanical test finding with BIC value.**

Statistics analysis of the removal torque value and bone implant contact (BIC) value for cpTi implants for study groups at eighth week interval were recorded, the torque value that needed to remove all the experimental implant was higher than the control, BIC value records same results. Findings also illustrate a significant difference in measurements for combination (VEGF & AV) in comparison to AV/VEGF group. While a non significant difference value was recorded for the comparison of VEGF with AV. The real domain of the expected torque force and BIC that needed in this period was shown in maximum and minimum value with 95% confidence interval for means and ANOVA test (tables 1,2,3).

**Histological findings**

In control group and at 1st week, osteoid tissue formation was detected at the apex of threads, then bone trabeculae was illustrated at the 2nd week. At eighth week a new lamellated bone showed in the base of implant bed that coalesce with bone thread.

Histological features for experimental group (VEGF) at 1st week showed osteoid deposition on the apex of the threads, then bone trabeculae mostly filled the threads at the 2nd week. At the eighth week an immature bone with osteon was observed.

In experimental group (AV) and at 1st week, osteoid tissue formation was detected at the apex of threads, then bone trabeculae filled most of implant bed was illustrated at the 2nd week. At eighth week an immature bone was observed.
Experimental group (combination VEGF&AV), at 1st week, showed an early formation of bone trabeculae on the apex thread, then it filled all the apex at the 2nd week. A mature bone with haversian system was illustrated at eighth week. Figure(2)

Figure(2) Implant views for studied groups shows developing threads at periods (1, 2 & 6 weeks)

1A: Shows threads (arrows) for control 1 week. H&Ex10

1B: Shows thread (arrow) with bone apposition for control 2 week. H&Ex10

1C: Shows thread (pink arrow) with bone formation extend to implant bed (green arrow) for control 8 week. H&Ex20

2A: Shows threads (pink arrows) with developing blood vessels (green arrow heads) at implant bed for VEGF group, at 1 week. H&Ex4

2B: Trabeculated bone (TB) fill most of thread for VEGF group, at 2 week. H&Ex10
2C : Immature bony threads for VEGF group, at 8week. H&Ex10

3A: Shows threads for AV group, at 1week. H&Ex10

3B: Bone trabecule (BT) mostly filled bed thread for AV group, at 2week. H&Ex10

3C: Immature bony threads for AV group, at 8week. H&Ex10

4A: Shows threads for combination group, at 1week. H&Ex10

4B: Bone trabecule (BT) fill most of thread for combination group, at 2week. H&Ex10

4C: Mature bony threads for combination group, at 8week. H&Ex20

Table (1) Statistics analysis for Removal Torque test in different studied groups at 8th week measured periods.

<table>
<thead>
<tr>
<th>Torque-test (Ncm)</th>
<th>Period</th>
<th>Material</th>
<th>Groups</th>
<th>No implant</th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>Std. Error</th>
<th>95% Confidence Interval for Mean</th>
<th>Min.</th>
<th>Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eighth week</td>
<td>VEGF</td>
<td>Exp.VEGF</td>
<td>6</td>
<td>23.16</td>
<td>0.60</td>
<td>0.33</td>
<td>22.20 - 25.11</td>
<td>22.36</td>
<td>24.66</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AV</td>
<td>Exp.AV</td>
<td>6</td>
<td>22.16</td>
<td>0.89</td>
<td>0.44</td>
<td>21.33 - 24.45</td>
<td>20.65</td>
<td>24.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AV &amp;VEGF</td>
<td>Exp.AV &amp;VEGF</td>
<td>6</td>
<td>28.55</td>
<td>1.60</td>
<td>0.80</td>
<td>26.95 - 30.65</td>
<td>27.13</td>
<td>30.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>without control</td>
<td></td>
<td>6</td>
<td>17.88</td>
<td>0.79</td>
<td>0.39</td>
<td>16.45 - 19.22</td>
<td>16.88</td>
<td>18.95</td>
</tr>
</tbody>
</table>

Table (2) Statistics analysis for BIC test in different studied groups at 8th week measured period.

<table>
<thead>
<tr>
<th>BIC test</th>
<th>Period</th>
<th>Material</th>
<th>Groups</th>
<th>No implant</th>
<th>Mean</th>
<th>Std. Dev.</th>
<th>Std. Error</th>
<th>95% Confidence Interval for Mean</th>
<th>Min.</th>
<th>Max.</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Eighth week</td>
<td>VEGF</td>
<td>Exp.VEGF</td>
<td>6</td>
<td>23.24</td>
<td>1.13</td>
<td>0.23</td>
<td>22.20 - 24.22</td>
<td>22.22</td>
<td>24.58</td>
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<tr>
<td></td>
<td></td>
<td>AV</td>
<td>Exp.AV</td>
<td>6</td>
<td>25.22</td>
<td>1.01</td>
<td>0.66</td>
<td>23.55 - 26.44</td>
<td>23.66</td>
<td>27.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AV &amp;VEGF</td>
<td>Exp. AV &amp;VEGF</td>
<td>6</td>
<td>29.54</td>
<td>0.54</td>
<td>0.66</td>
<td>27.95 - 30.65</td>
<td>28.13</td>
<td>30.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td>without control</td>
<td></td>
<td>6</td>
<td>11.62</td>
<td>1.94</td>
<td>0.33</td>
<td>10.45 - 13.22</td>
<td>10.88</td>
<td>13.45</td>
</tr>
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</table>
Table (3) LSD after ANOVA test for removal torque and BIC in 8th weeks distributed among different groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group</th>
<th>Mean Diff.</th>
<th>Sig.</th>
<th>C.S. (*)</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>VEGF</td>
<td>-7.20</td>
<td>0.000</td>
<td>HS</td>
</tr>
<tr>
<td></td>
<td>AV</td>
<td>-6.88</td>
<td>0.000</td>
<td>HS</td>
</tr>
<tr>
<td></td>
<td>Combination</td>
<td>-5.0</td>
<td>0.000</td>
<td>HS</td>
</tr>
<tr>
<td></td>
<td>VEGF</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>AV</td>
<td>0.55</td>
<td>0.334</td>
<td>NS</td>
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<td></td>
<td>Combination</td>
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<td>0.020</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>AV</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Combination</td>
<td>-1.30</td>
<td>0.011</td>
<td>S</td>
</tr>
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<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>VEGF</td>
<td>-1.40</td>
<td>0.050</td>
<td>S</td>
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<tr>
<td></td>
<td>AV</td>
<td>-1.50</td>
<td>0.022</td>
<td>S</td>
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<tr>
<td></td>
<td>Combination</td>
<td>-5.04</td>
<td>0.000</td>
<td>HS</td>
</tr>
<tr>
<td></td>
<td>VEGF</td>
<td></td>
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<td>AV</td>
<td>0.43</td>
<td>0.676</td>
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<td></td>
<td>Combination</td>
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<td>0.040</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>AV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Combination</td>
<td>0.55</td>
<td>0.543</td>
<td>NS</td>
</tr>
</tbody>
</table>

*P≤0.001 : high significant, P≤0.05 : significant, P≥0.05: non significant

Discussion

Aloe Vera is a well known plant in Iraq. Because of its use in folk medicine as a wound-healing agent, and in cosmetic preparation, and even widely used by public Iraqi people as mask face and in burn condition. On other hand using of VEGF was documented to enhance healing. Therefore we involved using of Aloe Vera with VEGF with implantation and study their effect on bone healing activity and osseointegration.

The present study shows an early osteoid deposition in VEGF group as osteoid tissue formation. These results could be attributed to, that the traumatic site includes, stem cells differentiate into osteoblasts (Catharino et al., 2014)[17] that enhanced by exogenous vascular endothelial growth factor (VEGF) which has been implicated in angiogenesis, and the proliferative osteoblast and active osteocyte were included in deposition of collagen fiber. Same results was illustrated for AV group.

We found also an advance osseointegration sign with early bone maturation in experimental group and specifically combination group, moreover removal torque and BIC values were higher in combination group in comparison to AV, VEGF and control groups at the studied period.

These results may due to the followings

1. Action of Aloe Vera include: anti-inflammation, antimicrobials, healing promotion, and biological/ immunological modulation [12,18,19]

2. Aloe Vera stimulate hard tissue formation include bone [20,21].

3. Aloe Vera act as a bioactive molecule and scaffold for implant bed regeneration.[22,23]
4. Angiogenic potential of VEGF is effective to constitute an enhancement effect for developing bone. [24,25,26]

5. The present findings establish proof that the angiogenic activity of VEGF or the stimulation enhancement of AV is insufficiently potent as compared to combination of AV with VEGF to achieve therapeutic benefit.

Conclusions

Our data suggest that Aloe Vera could be a candidate biomolecule for osseointegration that affect on bone -implant interface tissue regeneration. And a combination of AV with VEGF represent a such a new strategy that might ultimately be applicable to enhance implant stability and integration.

Acknowledgment: This work was supported by Prof. Dr. Hasan Majdi, Dean of Al-Mustaqbal University College/ Babel, Iraq

Source of Funding- by ours

Conflicts of Interest: The author declares no conflict of interest.

Ethical Clearance- all work of this study had done according to the National Council’s guide for the care of laboratory animals.

References


Anthropometric Study of Nose Parameters in Iraqi Arabic and Kurdish Subjects (Possible Personal Identification Tool)

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Abstract

Background and Purpose: The human nose has been reported to differ in its anatomy and morphology between different racial and ethnic groups. Nasal anthropometric features when studied can provide useful information for reconstruction or esthetic surgical procedures and personal identification for forensic purposes. Method: A total of 311 subjects with age (19-23) years were divided according to gender, race (Arabic or Kurdish), and residence regions in Iraq (north, middle and south). Different nose parameters were measured for the subjects using digital Vernier caliper. Results: Comparing races, the nose height and nose length were significant between Arab and Kurds, nasal index showed (60, 60.6) for Arabic vs. Kurdish, respectively. Sexual dimorphism was significant in all the parameters obtained. The fine nose was the commonest among all subjects. Parameters analysis revealed high positive significant correlation among various parameters except the nasal index that showed negative correlation with nasal height and length. Conclusions: Nasal morphometric parameters were unique for each person and have variations between different race (Arabic and Kurdish), gender variation and the correlation among various parameters been recorded for Iraqi people. These morphometric evaluations are the key for standardization among Iraqis, and in comparison with different countries, this have great values in anatomical, surgical (aesthetic) and forensic aspects regarding personal identification.

Keywords: anthropometry, nasal dimension, nasal index, personal identification, Rhinoplasty

Introduction

Morphometric variations are noticed in facial features among different races [¹]. The human nose has been reported to differ in its anatomy and morphology between different racial and ethnic groups [²]. Measurements of different parameters of nose became very worthy in procedures related to nasal surgery and cosmetology [³]. Nasal anthropometric features when studied can provide useful information to the rhinoplastic surgeon especially when nasal reconstruction is performed for individuals who desire to change their nasal morphology [³, ⁴]. The nasal shape differs in human populations like various facial structures. For example, the interval between nasal wings had higher significant values in Asian and African when compared to European race [⁵]. The nasal index is one of the morphometric variables among individuals. It defined as the ratio of nasal width divided to nasal height multiplied by 100. It was revealed that the nasal index was the best index for distinguishing the different human ethnicities [⁶, ⁷].

Material and Method

In this present study, a total of 311 pharmacy students were recruited for the study, having age 18-22years, at Al-Rafidain University College, Baghdad and approved by ethical committee in the university. All the subjects were normal healthy residents of Iraq, 156 male and 155 female, the study was conducted during the period from September 2018 to February 2019. The study purposes were explained to all subjects and a written informed consent was obtained from each subject. Medical history and clinical examination were obtained, none of those enrolled for the study have history of craniofacial trauma, nose diseases, congenital anomalies or surgery of the nose. Subjects are classified according to race into Kurdish race and Arabic race. In addition, subjects are also classified into their different origin; from North, middle and south of Iraq. Measurements of parameters were obtained directly from the nose by a single investigator (to eliminate error), by using a digital Vernier’s caliper. Measurements were recorded
Anthropometric measurements were performed depending on the following anatomical landmarks: nasion (n), it is the midline point nasofrontal suture and the nasal root; subnasale (sn), it is the point just at middle of columella base; alar curvature (ac), the most lateral point that could be seen at curved base line of each ala; and pronasale (prn), the most protruding point at the nasal tip. The Anthropometric parameters that were measured include the following, and are illustrated in Figure 1:

1. Nose height (NH): distance from subnasale to nasion
2. Nose length (NL): from pronasale to nasion
3. Width of nose (WN): span from right curve of nasal alae to left one.
4. Right nostril diameter (RND) where maximum diameter of right nostril measured
5. Left nostril diameter where (LND) maximum diameter measurement was done.
6. Right alar curve pronasale (RACP) distance from right curve of ala to nasal tip
7. Left alar curve pronasale (LACP) distance from left curve of nasal ala to nasal tip
8. Nasal index was measured by calculating the nasal width divided by nasal height multiplied by 100.

Nose shape had been classified into five types according to nasal indices \[^{10}\]: 1. Very fine nose with nasal index <54.9 (Hyperleptorrhine), 2. Fine nose with nasal index =55.0-69.9 (Leptorrhine), 3. Medium nose when nasal index had a value between 70.0-84.9 (Mesorrhine), 4. Broad nose with nasal index = 85.0-99.9 (Platyrrhine) and 5. Very broad nose when nasal index >100 (Hyperplatyrrhine).

The collected data was statistically analyzed using SPSS software v.20. Values were expressed as mean ± standard deviation (SD). T test, one way ANOVA followed by post hoc test, chi square Fisher Exact and Pearson correlation test were used to compare the differences of parameters with significance value P<0.05.

Results

All different nasal parameters were recorded in Table 1. The parameters were recorded as mean ± SD, comparing the two races Arabic and Kurdish in one hand and comparing males and females on other hand. Sexual dimorphism was noted with male having higher values than their female counterparts, which were significant in all obtained parameters and nasal index (Table 1).

Table 1: Nose morphometry among races and gender (measurements in mm). The results were expressed as mean ± SD. Significant difference marked by * (P<0.05)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Race</th>
<th>Mean ± SD</th>
<th>Gender</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nose height (NH)</td>
<td>Arabic (200)</td>
<td>54.4 ± 5.9 *</td>
<td>Female (156)</td>
<td>51.3 ± 3.8 *</td>
</tr>
<tr>
<td></td>
<td>Kurdish (111)</td>
<td>52.6 ± 5.0</td>
<td>Male (155)</td>
<td>56.3 ± 6.1</td>
</tr>
<tr>
<td>Nose length (NL)</td>
<td>Arabic</td>
<td>48.1 ± 6.2 *</td>
<td>Female</td>
<td>45.2 ± 4.3 *</td>
</tr>
<tr>
<td></td>
<td>Kurdish</td>
<td>46.4 ± 5.0</td>
<td>Male</td>
<td>49.8 ± 6.3</td>
</tr>
<tr>
<td>Width of Nose (WN)</td>
<td>Arabic</td>
<td>32.5 ± 4.5</td>
<td>Female</td>
<td>30.2 ± 2.9 *</td>
</tr>
<tr>
<td></td>
<td>Kurdish</td>
<td>31.7 ± 3.2</td>
<td>Male</td>
<td>34.2 ± 4.2</td>
</tr>
<tr>
<td>Right nostril diameter (RND)</td>
<td>Arabic</td>
<td>21 ± 3.2</td>
<td>Female</td>
<td>19.3 ± 2.4 *</td>
</tr>
<tr>
<td></td>
<td>Kurdish</td>
<td>20.4 ± 2.9</td>
<td>Male</td>
<td>22.3 ± 3.0</td>
</tr>
<tr>
<td>Left nostril diameter (LND)</td>
<td>Arabic</td>
<td>20.2 ± 3.6 *</td>
<td>Female</td>
<td>18.6 ± 2.5 *</td>
</tr>
<tr>
<td></td>
<td>Kurdish</td>
<td>20 ± 2.8</td>
<td>Male</td>
<td>21.7 ± 3.4</td>
</tr>
<tr>
<td>RACP</td>
<td>Arabic</td>
<td>33.5 ± 5.2</td>
<td>Female</td>
<td>30.8 ± 3.0 *</td>
</tr>
<tr>
<td></td>
<td>Kurdish</td>
<td>32.6 ± 3.9</td>
<td>Male</td>
<td>35.5 ± 5.1</td>
</tr>
<tr>
<td>LACP</td>
<td>Arabic</td>
<td>33.5 ± 4.7 *</td>
<td>Female</td>
<td>30.8 ± 3.0 *</td>
</tr>
<tr>
<td></td>
<td>Kurdish</td>
<td>32.6 ± 3.5</td>
<td>Male</td>
<td>35.6 ± 4.1</td>
</tr>
<tr>
<td>Nasal Index (NI)</td>
<td>Arabic</td>
<td>60 ± 7.9</td>
<td>Female</td>
<td>59.2 ± 6.4 *</td>
</tr>
<tr>
<td></td>
<td>Kurdish</td>
<td>60.6 ± 7.2</td>
<td>Male</td>
<td>61.2 ± 8.7</td>
</tr>
</tbody>
</table>
Comparison of anthropometric nasal measurements between the two races, Arabic and Kurdish Iraqis, a statistically significant difference was found for only four parameters; nose height, nose length, LACP and LND. On the other hand, nasal index shows no significance between the two races (Table 1). Furthermore, the correlations of different parameters among Arabic, Kurdish and total subjects were analyzed and presented in Table 2.

Table 2: Pearson correlation for different parameters of nose among Arabic, Kurdish and total subjects.

<table>
<thead>
<tr>
<th>Correlations</th>
<th>Nasal Height</th>
<th>Nasal Length</th>
<th>Nasal Width</th>
<th>Right Nostril Diameter</th>
<th>Left Nostril Diameter</th>
<th>RACP</th>
<th>LACP</th>
<th>Nasal Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal Height</td>
<td>total 1.0</td>
<td>0.8</td>
<td>0.4</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>0.5</td>
<td>-0.4</td>
</tr>
<tr>
<td>Arabic</td>
<td>1.0</td>
<td>0.9</td>
<td>0.4</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>0.6</td>
<td>-0.4</td>
</tr>
<tr>
<td>Kurdish</td>
<td>1.0</td>
<td>0.7</td>
<td>0.3</td>
<td>0.2</td>
<td>0.3</td>
<td>0.5</td>
<td>0.3</td>
<td>-0.6</td>
</tr>
<tr>
<td>Nasal Length</td>
<td>total 0.8</td>
<td>1.0</td>
<td>0.3</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>0.5</td>
<td>-0.3</td>
</tr>
<tr>
<td>Arabic</td>
<td>0.9</td>
<td>1.0</td>
<td>0.3</td>
<td>0.3</td>
<td>0.4</td>
<td>0.4</td>
<td>0.5</td>
<td>-0.3</td>
</tr>
<tr>
<td>Kurdish</td>
<td>0.7</td>
<td>1.0</td>
<td>0.3</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>0.4</td>
<td>-0.3</td>
</tr>
<tr>
<td>Nasal Width</td>
<td>total 0.4</td>
<td>0.3</td>
<td>1.0</td>
<td>0.7</td>
<td>0.8</td>
<td>0.5</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Arabic</td>
<td>0.4</td>
<td>0.3</td>
<td>1.0</td>
<td>0.8</td>
<td>0.8</td>
<td>0.5</td>
<td>0.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Kurdish</td>
<td>0.3</td>
<td>0.3</td>
<td>1.0</td>
<td>0.7</td>
<td>0.7</td>
<td>0.6</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Right Nostril Diameter</td>
<td>total 0.3</td>
<td>0.3</td>
<td>0.7</td>
<td>1.0</td>
<td>0.9</td>
<td>0.6</td>
<td>0.7</td>
<td>0.5</td>
</tr>
<tr>
<td>Arabic</td>
<td>0.3</td>
<td>0.3</td>
<td>0.8</td>
<td>1.0</td>
<td>0.9</td>
<td>0.6</td>
<td>0.7</td>
<td>0.5</td>
</tr>
<tr>
<td>Kurdish</td>
<td>0.2</td>
<td>0.3</td>
<td>0.7</td>
<td>1.0</td>
<td>0.8</td>
<td>0.7</td>
<td>0.5</td>
<td>0.4</td>
</tr>
<tr>
<td>Left Nostril Diameter</td>
<td>total 0.4</td>
<td>0.4</td>
<td>0.8</td>
<td>0.9</td>
<td>1.0</td>
<td>0.7</td>
<td>0.8</td>
<td>0.4</td>
</tr>
<tr>
<td>Arabic</td>
<td>0.4</td>
<td>0.4</td>
<td>0.8</td>
<td>0.9</td>
<td>1.0</td>
<td>0.7</td>
<td>0.8</td>
<td>0.5</td>
</tr>
<tr>
<td>Kurdish</td>
<td>0.3</td>
<td>0.4</td>
<td>0.7</td>
<td>0.8</td>
<td>1.0</td>
<td>0.8</td>
<td>0.6</td>
<td>0.3</td>
</tr>
<tr>
<td>RACP</td>
<td>total 0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.6</td>
<td>0.7</td>
<td>1.0</td>
<td>0.7</td>
<td>0.1</td>
</tr>
<tr>
<td>Arabic</td>
<td>0.5</td>
<td>0.4</td>
<td>0.5</td>
<td>0.6</td>
<td>0.7</td>
<td>1.0</td>
<td>0.8</td>
<td>0.1</td>
</tr>
<tr>
<td>Kurdish</td>
<td>0.5</td>
<td>0.5</td>
<td>0.6</td>
<td>0.7</td>
<td>0.8</td>
<td>1.0</td>
<td>0.6</td>
<td>0.1</td>
</tr>
<tr>
<td>LACP</td>
<td>total 0.5</td>
<td>0.5</td>
<td>0.7</td>
<td>0.7</td>
<td>0.8</td>
<td>0.7</td>
<td>1.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Arabic</td>
<td>0.6</td>
<td>0.5</td>
<td>0.7</td>
<td>0.7</td>
<td>0.8</td>
<td>0.8</td>
<td>1.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Kurdish</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
<td>1.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Nasal Index</td>
<td>total -0.4</td>
<td>-0.3</td>
<td>0.7</td>
<td>0.5</td>
<td>0.4</td>
<td>0.1</td>
<td>0.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Arabic</td>
<td>-0.4</td>
<td>-0.3</td>
<td>0.7</td>
<td>0.5</td>
<td>0.5</td>
<td>0.1</td>
<td>0.3</td>
<td>1.0</td>
</tr>
<tr>
<td>Kurdish</td>
<td>-0.6</td>
<td>-0.3</td>
<td>0.6</td>
<td>0.4</td>
<td>0.3</td>
<td>0.1</td>
<td>0.2</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Pearson correlation of various parameters revealed positive correlation among many parameters. The highest score was 0.9 and it noticed between Right and Left Nostril Diameter. In contrast, negative correlations were seen in nasal index with nasal height (-0.4, -0.4 and -0.6) for total, Arabic and Kurdish respectively. Another negative correlation was observed between nasal index and nasal length with same value for total, Arabic and Kurdish (-0.3) (Table 2). Nose shapes were calculated based on the number and percentage of nose shape by race (Arabic and Kurdish) and presented in Table 3. The most common shape in both races was the fine nose (68%) while the least frequency was the broad type (1%). No significant difference was observed between races and shape, indicating that the race does not have any effect on nose shape.

Table 3: Distribution of nose shape in different races, Arab and Kurd (Chi square)

<table>
<thead>
<tr>
<th>Shape</th>
<th>Arab</th>
<th>Kurd</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very fine nose</td>
<td>52</td>
<td>23</td>
<td>75 (24%)</td>
</tr>
<tr>
<td>Fine nose</td>
<td>130</td>
<td>82</td>
<td>212 (68%)</td>
</tr>
<tr>
<td>Medium nose</td>
<td>17</td>
<td>5</td>
<td>22 (7%)</td>
</tr>
<tr>
<td>Broad nose</td>
<td>1</td>
<td>1</td>
<td>2 (1%)</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>111</td>
<td>311</td>
</tr>
</tbody>
</table>

The results of the anthropometric analysis obtained among different regions of Iraq (north, middle, and south) are shown in Table 4. The middle region shows the highest parameters of all regions. One-way ANOVA test was performed to compare parameters among the three regions. A significant difference was seen only in nose height and nose length. Tukey post hoc test revealed the significance was only between the middle and north group and in both nose height and nose length (Table 4).

Table 4: Nose morphometry among different regions, north, middle and south (measurements in mm). The results were expressed as mean ±SD. One way ANOVA followed by post hoc Tukey analysis were used to compare the differences between groups. a, b, and c represent significantly different as compared to South, middle and north (P<0.05) respectively.

<table>
<thead>
<tr>
<th>Region</th>
<th>South N=93</th>
<th>Middle N=107</th>
<th>North N=111</th>
<th>Total N=311</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nose height</td>
<td>54 ± 5.3</td>
<td>54.7 ± 6.4 c</td>
<td>52.6 ± 5.0b</td>
<td>53.8 ± 5.7</td>
</tr>
<tr>
<td>Nose length</td>
<td>47.6 ± 5.4</td>
<td>48.5 ± 6.8 c</td>
<td>46.4 ± 5.0b</td>
<td>47.5 ± 5.8</td>
</tr>
<tr>
<td>Width of nose</td>
<td>32.2 ± 4.2</td>
<td>32.7 ± 4.8</td>
<td>31.7 ± 3.2</td>
<td>32.2 ± 4.1</td>
</tr>
<tr>
<td>Right nostril diameter</td>
<td>20.8 ± 3.1</td>
<td>21.1 ± 3.2</td>
<td>20.4 ± 2.9</td>
<td>20.7 ± 3.1</td>
</tr>
<tr>
<td>Left nostril diameter</td>
<td>20 ± 3.6</td>
<td>20.4 ± 3.6</td>
<td>20 ± 2.8</td>
<td>20.2 ± 3.4</td>
</tr>
<tr>
<td>RACP</td>
<td>33.3 ± 4.4</td>
<td>33.7 ± 5.738</td>
<td>32.6 ± 3.9</td>
<td>33.2 ± 4.8</td>
</tr>
<tr>
<td>LACP</td>
<td>32.9 ± 4.6</td>
<td>33.9 ± 4.8</td>
<td>32.6 ± 3.5</td>
<td>33.2 ± 4.3</td>
</tr>
<tr>
<td>Nasal Index</td>
<td>59.9 ± 6.9</td>
<td>60 ± 8.7</td>
<td>60.6 ± 7.2</td>
<td>60.2 ± 7.7</td>
</tr>
</tbody>
</table>
**Discussion**

The nasal morphometry is one of the significant features that characterize different races because it is at the center of the face. Different anthropological findings have described the nose as a signature indicating the ethnicity, race, age, and sex of an individual \cite{11, 12}. Morphometric assessments of parameters have clinical importance for nasal reconstruction after injury or for esthetic causes in addition to its importance in personal identification. Arabs had different race from Kurdish people that might help us to evaluate different race. Moreover there is variation in climate between north, middle and south of Iraq. According to our knowledge, the study of nose morphometry among Iraqis is the first study that carried out among our population, including the racial difference between Arabic and Kurdish. As presented above significant differences were noticed regarding nasal length and nasal height in both races. The dominant shape among subjects was the fine nose in both races. In addition, differences were noticed among nasal length, nasal height and nasal index. Some of the distinctive parameters that were carried out in this study are RACP and LACP. These two parameters assess distance of nasal tip and the nasal curve that provide indirect way to appreciate nasal deviation or not and the shorter distance means Ipsilateral nasal tip deviation. RND and LND parameters obtained above revealed RND and LND showed the high positive correlation only with NW and should be taken in consideration in reconstruction surgery.

Comparing our results with other results obtained from different studies, for instance among Nigerian populations \cite{13, 14}, there were lower values of nasal height and nasal length (44.1±3.7mm and 39.8±2.8mm) respectively, compared to our findings (53.8±5.7mm and 47.5±5.8 mm). However, nasal width and nasal index showed higher values (37.7±2mm and 86.1±8mm) compared to ours (32.2±4.1mm and 60.2±7.7mm) respectively. Both Nigerian studies showed similar findings to ours concerning sexual dimorphism, specifically there was significant difference in both gender regarding nasal height, nasal width and nasal index. Although, our results showed lower values of nasal width and nasal index compared to their results and higher values of nasal height. Conversely, on a recent Nigerian study, it stated lower values for nasal height, nasal length and nasal width compared to our results. Furthermore, it represented higher nasal indices for female and male (92.9, 98.9) respectively, and therefore broad nose is the dominant type, in contrast to ours, where the nasal indices for females and males were (59.2, 61.2) respectively, and fine nose is most common type. On the other hand, among Italian subjects nasal height, nasal length, nasal width and nasal index were 57.6±4mm, 49.3±4.8mm, 37.1±2.5mm and 64.9±6.9 respectively for adult male, while for females the same parameters were 54.3±4.2mm, 46.3±4.0mm, 35.3±2.6mm and 65.3±6.5 respectively \cite{15}. Our findings showed lower values for nasal height, nasal width and nasal index, with close values for nasal length.

**Conclusions**

Nasal morphometric parameters had different values for each parameter in different countries and had variations in regions within same country. Our results between Arabic and Kurdish, gender variation and the correlation among various parameters were recorded for Iraqi people subsequently these morphometric evaluations are the key for standardization among Iraqis, which have important impact for anthropologists, forensic and surgical procedures, by depending on values obtained from same community. In general, our study and many studies showed different nose parameters that emphasize the effects of race, sexual dimorphism, genetic and climate conditions on parameters.

**Source of Funding-** Self

**Ethical Clearance –** Not required

**Conflict of Interest:** None

**References**


Subjective Work Fatigue Due to Hot Work Climate  
(A Study on Indoor Production Workers of Ud King Rack  
Surabaya Indonesia)

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East Java, Indonesia

Abstract

One of physical factors contributing to the increasing number of work accidents and deaths is the hot working climate. One of the effects of hot working climate is work fatigue. The purpose of this study is to analyze the strength and direction of the relationship between the hot work climate and work fatigue in indoor production workers. This is an observational descriptive study using a cross sectional design. The sample was 33 workers. This study collected data of workers weight, workers height, pulse rate, Wet Bulb Globe Temperature, reaction timer and Subjective Self Rating Test. Data was analyzed using univariate and bivariate analysis.

The result showed that only working area of Oven 1 and Oven 2 that have the hot work climate exceeding the threshold limit value. All of 6 workers in those areas reported to experience subjective work fatigue. This study found that hot work climate and work fatigue has fairly strong and unidimensional relationship.

Keywords: hot work climate, work fatigue, subjective

Introduction

Hot work climate is one of the physical factors that are the cause of work-related illnesses, workplace accidents, and even death of workers. Work climate potentially causes health problems such as Prickly Heat, heat exhaustion, an increase of body temperature, heat stroke, heat cramps, increasing pulse rate, and dehydration1. Those health problems are because the body is not able to adapt to the work environment.

The hot working climate in Indonesia is regulated by the Threshold Limit Value (TLV) in the Minister of Manpower Regulation No. 5 of 2018 concerning Occupational Safety and Health, detailed at the Appendix point 1.

The work climate that exceeds the TLV will potentially disrupt workers health and comfort, and even can lead to death. An evidence of such cases was shown from the case found by NIOSH in 1992 to 2006. During those years, it found 423 workers death due to excessive exposure to the hot environment2. In addition, according to USA Bureau of Labor Statistics Report of 2015, there were 37 deaths and 2830 occupational injuries and diseases caused by exposure to hot environments 3.

One of the health problems that can occur is work fatigue. This is supported by the theory of “The Bucket Model” which explains that one of fatigue causes is climate4. According to Grandjean, in Setyawati’s book (2010), fatigue cannot be clearly explained or defined, but it can only be felt, however Grandjean (1995) defined fatigue as the feeling of tired and decreasing alertness. Therefore, this study focuses on the state of feeling tired. Feeling of fatigue is a subjective symptom in the form of...
uncomfortable and unpleasant feelings experienced by workers at work and after work.

Other than by climate, work fatigue are also caused by mental workload and physical workload, length of work, noise, ergonomics, emotional and organizational demands and personal factor.

There are researches that support the theory that subjective work fatigue is associated with a hot work climate. Research by Yuli Suryaningtyas and Noeroel Widajati (2017) on Work Climate and Nutritional Status with Work Fatigue on Workers in Ballast Tanks at Ship Repair Section PT. X Surabaya found that there is a moderate relationship with a positive direction between work climate and fatigue. The measurement of fatigue of their research was carried out using a subjective method, namely the KAUPK2 questionnaire. In this study, it found that the WBGT index in Ballast Tanks exceeded the TLV of the permissible work climate. It also found that most of workers felt very tired.

Based on the background above, it was encouraging to conduct a scientific study and research on the relationship of hot work climate and subjective work fatigue in Indoor Production workers UD King Rack Surabaya Indonesia.

**Material and Method**

This study is an observational study as the researchers did not provide special treatment and intervention to workers. The research was carried out with a cross sectional method, namely a one-time approach, where identification of causes and effects was carried out at the same time. This study was descriptive research in accordance with the nature of the problem and analysis of the data. This study used total population sample. Therefore, subjects of this research were all workers (33 workers) who worked in the indoor production section of UD King Rack Surabaya Indonesia.

This study aimed to determine the strength and direction of the relationship of hot work climate and subjective work fatigue. The independent variable in this study is the hot work climate and the dependent variable is subjective work fatigue. To determine the relationship strength relationship between the two variables, the theory of Sarwono (2006) was used. It categorized the strength into six categories based on the analysis results:

1. 0 = no correlation
2. 0-0,25 = weak
3. 0,25- 0,5 = moderate
4. 0,5-0,75 = strong
5. 0,75-0,99 = very strong
6. 1 = perfect.

The direction was concluded by the positive and negative sign of the correlation coefficient.

**RESULT**

A. Characteristics of Indoor Production Section Workers of UD King Rack Surabaya Indonesia

Table 1. Characteristic distribution of Indoor Production Section Workers of UD King Rack Surabaya Indonesia 2019

<table>
<thead>
<tr>
<th>Individual characteristics</th>
<th>Category</th>
<th>Total</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male</td>
<td>33</td>
<td>100</td>
</tr>
<tr>
<td>Age</td>
<td>20-30 years</td>
<td>28</td>
<td>84,8</td>
</tr>
<tr>
<td></td>
<td>31-40 years</td>
<td>2</td>
<td>6,1</td>
</tr>
<tr>
<td></td>
<td>&gt; 40 years</td>
<td>3</td>
<td>9,1</td>
</tr>
<tr>
<td>Years of service</td>
<td>&lt;5 years</td>
<td>16</td>
<td>48,5</td>
</tr>
<tr>
<td></td>
<td>5-10 years</td>
<td>13</td>
<td>39,4</td>
</tr>
<tr>
<td></td>
<td>&gt;10 years</td>
<td>4</td>
<td>12,1</td>
</tr>
<tr>
<td>Smoking habit</td>
<td>Yes</td>
<td>28</td>
<td>84,8</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>5</td>
<td>15,2</td>
</tr>
<tr>
<td>Body Mass Index (BMI)</td>
<td>Thin</td>
<td>9</td>
<td>27,3</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>21</td>
<td>63,6</td>
</tr>
<tr>
<td></td>
<td>Excess weight</td>
<td>2</td>
<td>6,1</td>
</tr>
<tr>
<td></td>
<td>Obesity</td>
<td>1</td>
<td>3,0</td>
</tr>
</tbody>
</table>

Table 1 shows that most of workers in the Indoor Production section 2019 are male aged 20 – 30 years. Most of them work for less than 5 years. They have smoking habit and their Body Mass Index (BMI) is normal.
B. Result of Hot Work Climate in the Indoor Production Section Workers of UD King Rack Surabaya Indonesia

The measurement of hot work climate was carried out in all indoor areas of UD King Rack Surabaya. The measurement was carried out using a tool called Heat Stress Apparatus from Quest Type Temp 36.

Table 2. Result of Hot Work Climate Measurement in Indoor Production Section of UD King Rack Surabaya Indonesia

<table>
<thead>
<tr>
<th>Location</th>
<th>Sb</th>
<th>Sk</th>
<th>Sg</th>
<th>WBGT</th>
<th>RH</th>
</tr>
</thead>
<tbody>
<tr>
<td>(°C)</td>
<td>(°C)</td>
<td>(°C)</td>
<td>(°C)</td>
<td>(°C)</td>
<td>(%)</td>
</tr>
<tr>
<td>oven 1</td>
<td>27,1</td>
<td>33,1</td>
<td>37</td>
<td>30,2</td>
<td>59,3</td>
</tr>
<tr>
<td>oven 2</td>
<td>27,4</td>
<td>33,8</td>
<td>37,4</td>
<td>30,7</td>
<td>58</td>
</tr>
<tr>
<td>Electric welding area</td>
<td>27,1</td>
<td>35,3</td>
<td>37</td>
<td>30,1</td>
<td>57,6</td>
</tr>
<tr>
<td>GTAW area</td>
<td>27,3</td>
<td>35,1</td>
<td>37,2</td>
<td>30,3</td>
<td>55,3</td>
</tr>
</tbody>
</table>

Air flow speed : 0.1 - 1 (m/dt)

Table 2 shows that the highest WBGT index was in the oven 2 area of 30.7°C and the lowest was in the friction welding area of 28.8°C.

C. Measurement Results of Workload for Indoor Production Workers UD King Rack Surabaya Indonesia

To assess whether work climate meet the TLV is not only from the WBGT index in each area, but also from the workload and working time arrangements for every hour. Heavy workload will affect to the declining resilience of the worker’s body in facing the hot work environment. Therefore, if the workload is heavy, it is necessary to have a comfortable work climate for workers. The workload measurement used in this study was a 10-pulse method workload measurement. The measurement of pulse rate included the Resting Pulse Rate (RPR) and Working Pulse Rate (WPR).

Table 3. Workload Measurement Result of Workers in Indoor Production Section of UD King Rack Surabaya Indonesia

<table>
<thead>
<tr>
<th>Work area</th>
<th>Light</th>
<th>Medium</th>
<th>Heavy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>oven 1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>oven 2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Electric welding area</td>
<td>3</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>GTAW area</td>
<td>12</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>friction welding area</td>
<td>12</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>81.81</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 3 shows that 6 workers (18.2%) in the Indoor Production Area of UD King Rack Surabaya have very heavy workload with a pulse rate of 125-150 per minute from 10 pulse rate measurements. Most of the workers (81.81%) experienced light workload with a pulse rate of 75-100 per minute.

D. Determination of Suitability of Hot Work Climate in Indoor Production UD King Rack Surabaya Indonesia 2019 with Threshold Limit Value

Based on the measurement of work climate (WBGT), workload and working time arrangements, it can be concluded that hot work climate in each area is in accordance with or did not exceed the Threshold Limit Value determined by the Minister of Manpower Regulation No. 5 of 2018 concerning Occupational Safety and Health at the Work Environment. The following table shows the suitability of hot work climate in Indoor Production of Surabaya Indonesia UD King Rack in 2019 with Threshold Limit Value of the above regulation:

Table 4. The Suitability of Hot Work Climate in Indoor Production UD King Rack Surabaya Indonesia 2019 with the TLV

<table>
<thead>
<tr>
<th>No</th>
<th>Work area</th>
<th>WBGT(°C)</th>
<th>Workload</th>
<th>Work time/ hours</th>
<th>Keterangan</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>oven 1</td>
<td>30.2</td>
<td>Heavy</td>
<td>75-100%</td>
<td>Not Suitable</td>
</tr>
<tr>
<td>2</td>
<td>oven 2</td>
<td>30.7</td>
<td>Heavy</td>
<td>75-100%</td>
<td>Not Suitable</td>
</tr>
<tr>
<td>3</td>
<td>Electric welding area</td>
<td>30.1</td>
<td>Light</td>
<td>75-100%</td>
<td>Suitable</td>
</tr>
<tr>
<td>4</td>
<td>GTAW area</td>
<td>30.3</td>
<td>Light</td>
<td>75-100%</td>
<td>Suitable</td>
</tr>
<tr>
<td>5</td>
<td>friction welding area</td>
<td>28.8</td>
<td>Light</td>
<td>75-100%</td>
<td>Suitable</td>
</tr>
</tbody>
</table>

Table 4 shows that there were 2 areas that have a work climate that is not in accordance with the Threshold Limit Value in terms of the WBGT index, workload and working time arrangements for each hour. The two areas were oven area 1 with WBGT of 30.2°C and oven area 2 with WBGT of 30.7°C.

E. Result of Subjective Work Fatigue Measurement

In this study, work fatigue data was obtained from a subjective method measurements in the form of Subjective Self Rating Test questionnaire, consisting of 30 questions. The fatigue category is divided into 4 categories: not tired, mild fatigue, moderate fatigue, and severe fatigue. The following table shows the results of the work fatigue measurements:
Table 5. Result of Subjective Work Fatigue Measurement of Indoor Production Workers UD King Rack Surabaya Indonesia 2019

<table>
<thead>
<tr>
<th>Work area</th>
<th>Subjective Work Fatigue</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Not tired</td>
<td>Light</td>
<td>Medium</td>
<td>Heavy</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>oven 1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>oven 2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Electric welding area</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>66,7</td>
<td>1</td>
</tr>
<tr>
<td>GTAW area</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>50</td>
<td>6</td>
</tr>
<tr>
<td>Friction welding area</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>50</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
<td>0</td>
<td>14</td>
<td>42,4</td>
<td>19</td>
</tr>
</tbody>
</table>

Table 5 shows subjective work fatigue in 5 work areas. It shows that the majority of workers (57.6% or 19 workers) of UD King Rack Surabaya’s Indoor Production workers experience moderate fatigue.

F. Analysis Results of the Relationship between Hot Work Climate and Subjective Work Fatigue in Indoor Production Workers UD. Surabaya Indonesia King Rack 2019

The following table shows the results of the analysis of the relationship between hot work climate and subjective work fatigue:

Table 6. Analysis Results of the Relationship between Hot Work Climate and Subjective Work Fatigue in Indoor Production Workers UD. Surabaya Indonesia King Rack 2019

<table>
<thead>
<tr>
<th>Hot work climate</th>
<th>Subjective Work Fatigue</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Not tired</td>
<td>Light</td>
<td>Medium</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Suitable</td>
<td>0</td>
<td>0</td>
<td>14</td>
<td>51,9</td>
</tr>
<tr>
<td>Not suitable</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 6 shows that workers who are exposed to hot work climate exceeding the TLV experienced moderate fatigue. The fatigue was 2.07 times greater than the moderate fatigue experienced by workers with a hot work climate within the TLV. The result of data analysis shows that there was a moderate relationship between hot work climate and subjective work fatigue in the indoor area of UD King Rack Surabaya Indonesia. The correlation was positive and can be seen from the correlation coefficient of 0.375.

**Discussion**

Based on the analysis results, there were only 2 areas that have hot work climate index exceeding the Threshold Limit Value. It was area of oven 1 and oven 2. The relationship between hot work climate and subjective fatigue is quite strong. It was shown by the fact that workers of area oven 1 and oven 2 reported complaints both physically and motivationally, especially those who often experienced dizziness and dehydration. The complaint was caused by very high temperatures released by the oven with a temperature of 75°C to 90°C.

Direct exposure to the heat from the oven adds the heat burden felt by workers. Workers were often seen to stop working in order to drink and take a break while waiting for oven to process which takes 5 minutes. This indicates that the occurrence of worker fatigue. As the consequences, the fatigue can decrease worker work speed, make them to be more prone to errors and less productive, which lead to the increasing risk of negative safety or incidents. In fact, one study estimates work fatigue contributes to 13% of workplace injuries. During the interview, complaints to be most of workers reported were dizziness and thirst. Dizziness and dehydration are among the physical symptoms of fatigue.

Subjective work fatigue indicates that there is no worker experiencing severe fatigue. This is because workers have acclimatized to the existing temperature. They feel accustomed and report small number of complaints of continuous fatigue symptoms. This is in line with their working period in the oven area. Most of them have been working for more than 1 month while the acclimatization process only needs 5-7 days for workers to adapt the hot work climate with the longest of 12-14 days.

The relationship between hot work climate and subjective fatigue has a positive direction. This means that if the hot work climate increases, subjective work fatigue also increases.

This result is in line with research from Ridha Ramayanti (2018) entitled “Analysis of the Relationship between Nutritional Status and Work Climate with Fatigue in Catering in Surabaya Food Wisdom”. Ramayanti found that there is a moderate positive relationship between the work climate and work fatigue. The hot work climate at Catering Surabaya’s Food exceeded the TLV. Their workers experienced severe, moderate, and mild fatigue. Ramayanti (2018) also used the same subjective method, the Subjective Self Rating Test questionnaire from Japan’s Industrial Fatigue Research Committee (IFRC).

**Conclusion**

1. Workers in the Indoor Production Section of the UD King Rack Surabaya are all male. Most workers are 20-30 years old, with work periods of less than 5 years, have smoking habits, and have a normal MBI.

2. The highest WBGT index value is in the oven 2 area of 30.7°C and the lowest is in the friction welding area of 28.8°C. Hot work climate that is not in accordance with the Threshold Limir Value set by PERMENAKER No. 5 of 2018 is in the oven area 1 with WBGT of 30.2°C and oven area 2 with WBGT of 30.7°C. All workers in the areas where the hot work climate exceeded the TLV reported to have heavy workloads and to set the working time 75% -100% for every hour.

3. The result of subjective work fatigue indicates that all workers (100%) in the area exceeded the TLV of work climate reported moderate fatigue.

4. There is a positive moderate relationship between the hot work climate and subjective work fatigue

**Recommendation**

1. Control of heat sources or oven by isolation or limiting heat source exposure to other areas.

2. To manage the hot work climate with the addition supply of air conditioning devices such as fans

3. To provide quite cool rooms for body cooling equipped with drinking water supply for workers of oven 1 and 2.

4. To educate worker on the importance of
drinking 150-200 cc of water or equivalent to a glass of water for every 15 minutes -20 minutes.

5. To set working time 25% - 50% for oven 1 workers every hour, equivalent to 15 to 30 minutes working for every hour, and 20% - 25% for oven 2 workers, equivalent to a maximum of 15 minutes working time for every hour.

Conflict of Interest: All authors have no conflicts of interest to declare.

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Ethical Clearance: The study was approved by the institutional Ethical Board of the Public Health, Airlangga University.

References
Histopathological Assessment of Hepato-Nephrotoxicity Induced by Nano Copper Particles in Adult Male Albino Rats and the Potential Protective Effect of Alpha Lipoic Acid: A Chronic Study

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Abstract

Background: Copper nanoparticles (CNPs) have unique physical and chemical properties for this reason it was widely used in various medical and non-medical applications. This study aimed to study the toxic effect of copper nanoparticles on liver and kidney through histopathological examination of liver and kidney tissues and if there is improvement of hepato-nephrotoxicity after cessation of exposure and to study the potential protective effect of alpha lipoic acid (ALA) against hepato-nephrotoxicity induced by copper nanoparticles.

Finding: Histopathological examination of liver and kidney tissues in rats received CNPs showed significant alteration, cessation of exposure can cause slight improvement and ALA can cause significant improvement.

Conclusion: Copper Nanoparticles have hepato-nephrotoxic effect and ALA can be safely used as a protective agent against CNPs toxicity.

Keywords: Copper nanoparticles, Nanotoxicity, Hepatotoxicity, Nephrotoxicity, Histopathology.

Background

Nanotechnology is highly promising technology that is concerned with understanding the behavior of nanoparticles and its properties and how to control particles at nanoscale range.1

Copper nanoparticles have unique physical and chemical properties such as high electrical conductivity, high melting point, low electrochemical migration behavior.2

The increasing production and use of metal nanoparticles for different applications lead to many adverse effects on health.3

The main target organs for toxicity with CNPs are kidney, liver and blood. Liver is the main damaged organ.4

Alpha lipoic acid is a potent antioxidant. It has antioxidant activity in both fat and water-soluble mediums.5

There for the aim of this study is to identify the toxic effect of CNPs on liver and kidney through histopathological examination and to study the potential protective effect of ALA against hepato-nephrotoxicity induced by CNPs.
Methodology

Chemicals

1. Copper Nanoparticles

Copper Nano Particles (CNPs) 25 nm at 99.5% purity, mineral in nature and spherical in shape, was purchased from Sigma Aldrich, co. (St. Louis, Missouri, USA).

2. ALPHA LIPOIC ACID

Alpha lipoic acid (ALA) at 99% purity, was purchased from Sigma Aldrich, co. (St. Louis, Missouri, USA).

Study Design

The study design was approved from the local ethical committee of faculty of medicine, Cairo university and from Cairo University Institutional Animal Care and Use Committee (CU- IACUC), Medical Science Sector.

Animal grouping:

100 adult male albino rats of body weight 150–170 g was supplied and housed by the National Instituted of Ophthalmology. All animals have free access to water and food and exposed to 12 hours light/dark cycle. After acclimatization for 2 weeks, 100 adult male albino rats were randomized into four main groups and were treated daily for 90 consecutive days as follows:

Group I act as controls (30 rats):

Group I-A (negative control group of 10 rats),

Group I-B (10 rats): receive deionized water (2 ml) daily,

Group I-C (10 rats): receive olive oil (2 ml) daily.

Group II (30 rats):

Group II-A (20 rats): receive Copper Nano Particles (40 mg/kg bw) for 90 days,

Group II-B (follow up group of 10 rats): receive Copper Nano Particles (40 mg/kg bw) for 90 days then no treatment for the following 4 weeks.

Group III (20 rats): receive ALA (100 mg/kg bw) daily.

Group IV (20 rats): receive Copper Nano Particles (40 mg/kg bw) and ALA (100 mg/kg bw) as a prophylactic agent.

After 90 consecutive days following the last dose administration, rats were anaesthetized and sacrificed by decapitation. The follow up group were allowed for recovery with no treatment for 30 days then scarified as before.

Dose Selection and Preparation of Oral Suspension

• Copper Nanoparticles Dose:

LD50 for CNPs is 413 mg/kg body weight. The selected dose of CNPs was 1/10 LD50 which induce biochemical alteration in rats without morbidity. Stock suspension of CNPs was prepared by dispersing CNP powder in deionized water followed by vigorous vertexing and sonication.

• Alpha lipoic Acid Dose:

The selected dose for ALA is (100 mg/kg bw) was reported to be hepatoprotective in rats. Stock of ALA oral suspension was prepared by dissolving ALA in olive oil to improve its absorption by vigorous vertexing and sonication.

At the End of the Study Period:

After 90 consecutive days following the last dose administration, rats were sacrificed. The follow up group were allowed for recovery with no treatment for 30 days then scarified.

Histopathological Observation:

liver and kidney from each rat were cut rapidly, fixed in neutral purified formalin (10%), then dehydrated with grades of ethanol (70,80,90,95 and 100%). Dehydration is then followed by clearing the samples with 2 cycles of xylene. Samples were then impregnated with 2 changes of molten paraffin wax, then embedded and blocked out. Paraffin sections (4-5 μm) were stained in hematoxylin and eosin and get examined under light microscope under high power (X100).

Stained sections of all groups were examined for:

1- Liver: alteration of architecture, portal inflammation, hepatocytes and for the presence of degeneration, necrosis and portal fibrosis.
2- **Kidney**: alteration of architecture of the cortex and medullary segments and the structure of glomeruli as well as proximal and distal tubules.

**Statistical Analysis**

Data were coded and entered using the statistical package of Social Sciences (SPSS). Comparisons between groups were done using analysis of variance (ANOVA) with multiple comparisons post hoc test.\(^8\) For comparing categorical data, Chi square \((\chi^2)\) test was performed.\(^9\)

**Finding**

Liver Histopathological Examination of the Studied Groups:

1. **Histopathological Examination of Group 1 & Group 3:**
   
   Histopathological examination of sections of liver from rats of group 1 and group 3 showed normal hepatic parenchyma with preserved lobular architecture, normal arrangement and structure of the hepatocytes. The portal tract showed no inflammatory cells. No congestion was seen, and central vein was within normal (figures 1).

2. **Histopathological Examination of Group 2A:**
   
   Histopathological examination of sections of liver from rats of group 2A showed severe histopathological alterations characterized by dilatation of central vein with disruption of lobular architecture and disorganization of hepatic cords as well as necrobiotic changes in the form of vacuolar degeneration with vesicular nuclei of the hepatocyte and fat accumulation; steatosis. The portal tract is dilated with inflammatory exudate. Periportal fibrosis was detected and the central vein was dilated (figure 1).

3. **Histopathological Examination of Group 2B:**
   
   Histopathological examination of sections of liver from rats of group 2B revealed mild ballooning of the hepatocytes, however the portal area is dilated and showed inflammatory cell infiltrate, the central vein was dilated congested. The hepatocytes showed some disorganization (figure 2).

4. **Histopathological Examination of Group 4:**
   
   Histopathological examination of sections of liver from rats of group 4 revealed that portal tracks appeared normal in some sections other sections showed minimal to mild inflammation in occasional portal tracts, the central vein is of normal caliber and is not congested. The hepatocytes showed minimal to absent vacuolar changes (figure 2).

**Comparison Between Studied Groups using Chi-Square Tests:**

Regarding severity of lesion in the four main studied group it was found that, 35% of liver sections of group 2A showed severe lesion and 54% of sections showed moderate lesion while 10% of liver sections of group 2B showed sever lesion and 40% was moderately affected compared to the three control groups 1A,1B & 1C as well as group 3 which were normal. While 65% of group 4 liver section were normal and 35% showed mild lesion with statistically significant difference between the 4 main groups (p value <0.001) (figure 3).

Renal Histopathological Examination of the Studied Groups:

1. **Histopathological Examination of Group 1 & Group 3:**
   
   Histopathological examination of group 1 & group 3 showed normal appearance of the glomeruli with normal cellularity. The tubules are not dilated with the lining cells are of normal appearance and the interstitial tissues showed no inflammation or congested blood vessels (figure 4).

2. **Histopathological Examination of Group 2A:**
   
   Histopathological examination of group 2A showed swollen glomeruli with thickened basement membrane. Both the glomeruli and the interstitial blood vessels are congested. Foci of fibrosis are seen. The tubular lining showed degenerative changes (figure 4).

3. **Histopathological Examination of Group 2B:**
   
   Histopathological examination of group 2B showed swollen glomeruli with thickened basement membrane with congested both glomerular and interstitial blood vessels however, the tubular lining showed no degenerative changes and there was no fibrosis seen (figure 4).
4. Histopathological Examination of Group 4:

Histopathological examination of group 4 showed thin basement membrane of the glomeruli. Both the tubules and interstitial tissues showed no pathological changes apart from minimal degenerative changes of the tubular lining (figure 4).

Comparison Between Studied Groups using Chi-Square Tests:

Regarding severity of lesion in the four main studied group it was found that, 40% of renal sections of both members of group 2 (2A & 2B) showed moderate lesion and 60% of sections showed mild lesion compared to the three control groups 1A, 1B & 1C as well as group 3 which were normal. While 75% of group 4 liver section were normal and 25% showed mild lesion with statistically significant difference between the 4 main groups (p value <0.001) (figure 5).

Pairwise Comparison Between Studied Groups

Using Post hoc Pairwise Comparisons between group 1, group 2A, 2B, group 3 and group 4 in histopathological examination of liver sections there was statistically significant difference between group 2 with groups 1, 3, 4 (p value <0.001) and group 4 with groups 2A & 2B (p value <0.001) while there was no statistical significant difference between groups 2A and 2B.

Using Post hoc Pairwise Comparisons between group 1, group 2A, 2B, group 3 and group 4 in histopathological examination of renal sections there is statistical significant difference between group 2 with groups 1, 3, 4 (p value <0.001) and group 4 with groups 2A & 2B (p value <0.001) while there was no statistical significant difference between groups 2A and 2B.

Figure 1: Sections of liver a) group 3 with normal appearance, central vein (the star) within normal b) group 2A showed vesicular nuclei of the hepatocyte with the portal tract is dilated showed inflammatory exudate (the arrow), c) group 2A showed dilated central vein (the star). d) group 2A showed mildly dilated congested central vein (the star). The hepatocytes showed disorganization, with hydropic changes and ballooning (the circle) and steatosis (the empty arrow). X100 H&E.
Figure 2: Sections from the liver: a) group 2B showed mild ballooning of the hepatocytes, the portal area is dilated and showed inflammatory cell infiltrate (the arrow), b) group 2B with dilated blood vessel (the star), with the portal tract is dilated with inflammatory exudate (the black arrow). The hepatocytes showed some vascular degeneration, c) group 4 showed minimal to mild inflammation in occasional portal tracts (the black arrow), the central vein is of normal caliber and is not congested. The hepatocytes showed minimal to absent vacuolar changes, d) group 4 showed within normal portal tracts (the black arrow), the hepatocytes showed no hydropic degeneration (the circle), the central vein is of normal caliber and is not congested (the star) x100 H&E.

Figure 3: Histopathological grading of liver sections in the 4 studied groups.
Figure 4: Section from kidney a) group 3; showed normal appearance of the glomeruli with normal cellularity (the arrow). The tubules and the lining cells are of normal appearance, b) group 2A showed swollen glomeruli with thickened basement membrane (the black arrow). Both the glomerular and the interstitial blood vessels are congested (the star). Foci of fibrosis are seen (empty arrow). The tubular lining showed degenerative changes, c) group 2B showed swollen glomeruli with thickened basement membrane (the arrow) with congested both glomerular and interstitial blood vessels, d) group 4 showed thin basement membrane of the glomeruli (the black arrow). Both the tubules and interstitial tissues showed no pathological changes apart from minimal degenerative changes of the tubular lining. X100 H&E.

Figure 5: Histopathological grading of renal sections in the 4 studied groups.
**Discussion**

Histopathological alteration of liver tissue could be explained by accumulation of CNPs in the liver which can cause discontinuous endothelia that allow the passage of NPs from the blood into the liver parenchyma. This accumulation is responsible for the sever histopathological alterations.10

In accordance with our study, CNPs caused various histopathological alterations in the liver tissues and the hepatocytes.6,11

The protective effect of ALA against liver toxicity could be explained as ALA was considered as chelator compound as it can chelate metals ions.12 ALA has a protective effect of on kidney and the histopathological result showed marked improvement in the group of rats received ALA.13

CNPs caused severe damage to the kidney tissues as the strong ionization potential of copper ions lead to its accumulation in the renal tissue which lead to inflammation in the renal tissues.14 In accordance, CNPs caused widespread tubular necrosis15 and can cause damage to the renal tissue14.

**Conclusion and Recommendations**

- CNPs caused histopathological alterations in liver and kidney tissues.
- Discontinuation of CNPs exposure in the follow up group caused non-significant improvement in liver tissues and did not improve the kidney tissues.
- Alpha lipoic acid can cause improvement in the histopathological changes caused by CNPs toxicity.
- Results of this study recommend periodic medical examination as well as Liver and kidney function tests in case of continuous exposure to CNPs.
- In case of deterioration of liver functions due to continuous exposure to CNPs; a period of cessation of exposure for at least 4 weeks is recommended.
- Alpha lipoic acid can be safely used as a prophylactic agent in case of Nano copper exposure.
- Confirmation of this study results regarding the period of cessation of exposure to CNPs is needed with prolongation of the follow up period.

**Conflict of Interest:** The authors declared that they have no Conflict of interests.

**Funding:** No funding source for this article

**Ethics Approval:** The study work was conducted after the approval of Ethical Committee, Faculty of medicine, Cairo University and the approval of Cairo University Institutional Animal Care and Use Committee (CU- IACUC), Medical Science Sector.

**References**


Safe Concentration of Benzene Exposure to Worker’s in Gas Station at the Area of Diponegoro University, Semarang

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Abstract

Benzene is one of the aromatic hydrocarbon compounds that are widely used in the industrial sector. Benzene exposure in the work environment has been set a threshold value. Benzene exposure in humans can provide health effects especially disrupting the central nervous system, hematopoietic system, and immune system. Acute effects can include laryngeal irritation, dizziness, pallor, shortness of breath, headache, fatigue, drowsiness, and fainting. While the chronic effects can be cancer.

This research is a study with an environmental health risk analysis approach with the aim to assess and make predictions that will occur due to exposure to benzene in public fuel station (SPBU) officers around the area of Diponegoro University, Semarang. The Environmental Health Risk Analysis (ARKL) approach consists of several steps, namely hazard identification, response dose analysis, exposure analysis, and risk characteristics.

The results of the measurement of benzene concentrations in gas stations around the Diponegoro University in Semarang at point 1 were 0.38 ppm and at point 2 was 0.51 ppm. So that the concentration is still below the Threshold Value (NAB) according to the Minister of Manpower Regulation Number 5 of 2018. Based on manual calculations for safe limits benzene concentration is obtained 0.08538 mg / m³ or 0.02672 ppm. This means that the highest concentration of benzene in the work environment is 0.51 ppm, with an average body weight of 55.67 kg, height of 159 cm with a working time of 8 hours, and safe concentration so as not to cause a non-carcinogenic risk is 0.08538 mg / m³ or 0.02672 ppm.

Keyword: Benzene, Safe Concentration, Gas Station

Introduction

Besides being used in industry, benzene is found to be widely available in our daily lives. Benzene is produced by distillation of coal or crude oil. This material is used as one of the raw materials in the production of many aromatic compounds such as styrene, phenol, cyclohexane, and nitrobenzen, also in drugs, pesticides, and detergents. Sometimes benzene is also used as an extraction solvent. This material can also be found in solvents for wax, resin, rubber, plastics, film, and glue paint. In recent years the use of solvents has been restricted or banned in many countries due to its toxicity. Benzene is present in gasoline (petroleum), toluene and xylene in impure form.1

Around 1990 benzene was used as an indicator of exposure to gasoline fuel, especially for workers at gas stations. The volume of benzene in gasoline fuels ranges from 2 - 6% in the Nordic country. The allowed working time is 8 hours a day, the gas station staff in the Nordic are exposed to benzene around 0.5-1 mg / m³. Exposure to gasoline vapor at gas stations, especially when refueling gasoline into the car tank. Filling 30 liters containing 5% volume of benzene into the car, there is around 700 mg of benzene inhaled. The total concentration of hydrocarbons in the air during the
process of refueling gasoline is 10 to 100 times benzene. Gas station staff can also be exposed to vehicle gas emissions, including polycyclic aromatic hydrocarbons, aldehydes, and 1,3-butadiene.

According to WHO, one of the sources of benzene in ambient air comes from benzene evaporation in fueling stations. Benzene concentrations in ambient air are estimated to be inhaled and exposed to fuel station workers of 0.12 ppm. Benzene exposure in humans can provide health effects especially disrupting the central nervous system, hematopoietic system, and immune system. Acute effects can include laryngeal irritation, dizziness, pallor, shortness of breath, headache, fatigue, drowsiness, and fainting. While the chronic effects can be cancer.

According to the Agency for Toxic Substances and Disease Register (ATSDR), hazardous and toxic chemicals contained in oil content are benzene, toluene, xylene, ethylene, TPH (Total Petroleum Hydrocarbon), and Polycyclic Aromatic Hydrocarbon (PAHs). Of the six chemicals, benzene exposure has a very serious impact on health.

Population growth in the city of Semarang has increased in accordance with data from the Central Statistics Agency (BPS) in 2013 of 1,672,999 people. This was also due to population growth in each sub-district in Semarang City, especially Tembalang and Banyumanik Districts. Where the two sub-districts are several universities, one of which is Diponegoro University. This made the attraction of migrants from various regions to come to the two sub-districts. As in the 2013 Central Bureau of Statistics (BPS) data, the population in Tembalang and Banyumanik Subdistricts was 147,564 people and 130,494 inhabitants.

Every day the gas station around the area of Diponegoro University fills 24,000 liters of fuel. The fuel in the Gas Station causes a strong odor that can be inhaled and entered into the human body, one of them is a gas station officer who maintains fuel installations. The smell of these fuels contains benzene organic compounds. Other studies show that the results of benzene exposure in the air to the refueling station staff are 0.23 ppm or 0.73 mg / m3.

Benzene exposure in the work environment has been set a threshold value. In Indonesia the AMbang Limit (NAV) of benzene is 0.5 ppm. Use the Regulation of the Minister of Manpower and Transmigration number PER / 13 / MEN / X / 2011 concerning the Physical and Chemical Factor Threshold Values in the workplace and according to NIOSH, the threshold value of benzene is 0.1 ppm.

Fuel oil is a mixture of more than 500 volatile hydrocarbon compounds and benzene is a hydrocarbon compound which is a major concern in studies that explain health problems due to benzene exposure. The population of workers who work in industries that produce or use benzene can be exposed to the highest exposure levels. ATSDR (2007) states that the main exposure route occurs through inhalation, although dermal exposure and oral exposure may also occur.

Material and Method
This research is a research with an environmental health risk analysis approach with the aim of assessing and making predictions that will occur due to benzene exposure in gas station officers around the Diponegoro University Semarang area. The Environmental Health Risk Analysis (ARKL) approach consists of several steps, namely hazard identification, response dose analysis, exposure analysis, and risk characteristics.

The population of this study were all officers at four gas stations around the area of Diponegoro University. The number of officers at the four gas station around the Diponegoro University area is 78 people and the study sample was calculated using the Slovin formula.

The sampling technique used purposive sampling based on certain considerations made by researchers with the inclusion criteria was officers who had direct contact with benzene sources, male and/or female, worked on the morning and afternoon shifts and worked ≥ 1 year. Measurement of benzene concentrations in ambient air that is inhaled directly by gas station officers using a personal dust sampler and coconut shell charcoal. Secondary data consists of workplace administrative data, such as number of workers, length of service, type of fuel, work shift schedule. Study of intake calculations, non carcinogenic, carcinogenic, dose response values, and technical methods of Environmental Health Risk Analysis (ARKL).

Findings
A. Worker Characteristics, Worker’s Body Surface Area and Worker’s Respiratory Rate
The characteristics of the workers in this study included the body weight and working time of 27 workers.
in the Ciputat area gas station area. Based on Table 1, it is known that the highest body weight of workers in the Ciputat area gas station area is 80 kg, the lowest weight is 44 kg, and the average body weight is 57.73 kg. The duration of work in a day is 8 hours. Whereas for height use the average value of Indonesian adult male height which is 159 cm.

Based on data on worker weight and height of workers, the body surface area and the rate of respiration of workers can be calculated using the following formula.

1. The surface area of the worker’s body

\[ BSA = \sqrt{\frac{W \cdot h}{3600}} \]

2. Worker breathing rate:

\[ BR = \frac{5.3 \ln - 6.9}{24} \]

<table>
<thead>
<tr>
<th>Worker</th>
<th>W (kg)</th>
<th>H (cm)</th>
<th>BSA (m²)</th>
<th>BR (m³/hour)</th>
<th>t (hour/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>159</td>
<td>1.32916</td>
<td>0.52713</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>43</td>
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<td>1.3781</td>
<td>0.5431</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>44</td>
<td>159</td>
<td>1.39403</td>
<td>0.54818</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>52</td>
<td>159</td>
<td>1.51548</td>
<td>0.58507</td>
<td>8</td>
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<tr>
<td>5</td>
<td>46</td>
<td>159</td>
<td>1.42537</td>
<td>0.55799</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>47</td>
<td>159</td>
<td>1.44078</td>
<td>0.56274</td>
<td>8</td>
</tr>
<tr>
<td>7</td>
<td>49</td>
<td>159</td>
<td>1.47111</td>
<td>0.57194</td>
<td>8</td>
</tr>
<tr>
<td>8</td>
<td>44</td>
<td>159</td>
<td>1.39403</td>
<td>0.54818</td>
<td>8</td>
</tr>
<tr>
<td>9</td>
<td>47</td>
<td>159</td>
<td>1.44078</td>
<td>0.56274</td>
<td>8</td>
</tr>
<tr>
<td>10</td>
<td>49</td>
<td>159</td>
<td>1.47111</td>
<td>0.57194</td>
<td>8</td>
</tr>
<tr>
<td>11</td>
<td>50</td>
<td>159</td>
<td>1.48605</td>
<td>0.57641</td>
<td>8</td>
</tr>
<tr>
<td>12</td>
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<td>159</td>
<td>1.40979</td>
<td>0.55314</td>
<td>8</td>
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<tr>
<td>13</td>
<td>54</td>
<td>159</td>
<td>1.54434</td>
<td>0.5934</td>
<td>8</td>
</tr>
<tr>
<td>14</td>
<td>58</td>
<td>159</td>
<td>1.60052</td>
<td>0.60918</td>
<td>8</td>
</tr>
<tr>
<td>15</td>
<td>59</td>
<td>159</td>
<td>1.61426</td>
<td>0.61296</td>
<td>8</td>
</tr>
<tr>
<td>16</td>
<td>60</td>
<td>159</td>
<td>1.62788</td>
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<td>1.69435</td>
<td>0.63434</td>
<td>8</td>
</tr>
<tr>
<td>18</td>
<td>62</td>
<td>159</td>
<td>1.65479</td>
<td>0.62391</td>
<td>8</td>
</tr>
<tr>
<td>19</td>
<td>70</td>
<td>159</td>
<td>1.75831</td>
<td>0.65071</td>
<td>8</td>
</tr>
</tbody>
</table>
The results of the analysis of the calculation of body surface area and worker respiratory rate according to Table 1 show that the average body surface area of workers is 1.56 m² and the average respiration rate of workers is 0.59 m³/hour.

B. Benzene Concentration

The results of the measurement of benzene concentration at both points in the Ciputat region gas station environment showed the same results, which was equal to 0.58 mg/m³ (0.18 ppm).

Table 2. Distribution of Benzene Concentration in Gas Station Around the Area of Diponegoro University, Semarang

<table>
<thead>
<tr>
<th>Measurement Location</th>
<th>Benzene Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location 1</td>
<td>0.38</td>
</tr>
<tr>
<td>Location 2</td>
<td>0.51</td>
</tr>
<tr>
<td>Average</td>
<td>0.44</td>
</tr>
</tbody>
</table>

Based on the results of measurements made, the concentration of benzene in the work environment at the gas stations around the Diponegoro University Semarang area is 0.44 ppm. This benzene concentration is below the threshold value (NAB) of 0.5 ppm in accordance with the provisions of the Minister of Manpower Regulation Number 5 of 2018 concerning Occupational Safety and Health at the Work Environment. However, the benzene concentration was above the Minimum Risk Level (MRL), the level of exposure to inhalation benzene determined by ATSDR, namely for acute exposure (≤14 days) = 0.009 ppm, moderate exposure (15-364 days) = 0.006 ppm, and chronic exposure (≥365 days) = 0.003 ppm.

C. Calculation of Human Km

The results of the Human Km calculation are shown in Table 3. Based on table 3, the average Human Km at the Gas Station Around the Area of Diponegoro University Semarang is 35.37.
Table 3. Results of Human Km Calculation for Workers at Gas Station Around the Area of Diponegoro University Semarang

<table>
<thead>
<tr>
<th>Worker</th>
<th>Human KM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30,0942</td>
</tr>
<tr>
<td>2</td>
<td>31,2023</td>
</tr>
<tr>
<td>3</td>
<td>31,5631</td>
</tr>
<tr>
<td>4</td>
<td>34,3127</td>
</tr>
<tr>
<td>5</td>
<td>32,2724</td>
</tr>
<tr>
<td>6</td>
<td>32,6213</td>
</tr>
<tr>
<td>7</td>
<td>33,3082</td>
</tr>
<tr>
<td>8</td>
<td>31,5631</td>
</tr>
<tr>
<td>9</td>
<td>32,6213</td>
</tr>
<tr>
<td>10</td>
<td>33,3082</td>
</tr>
<tr>
<td>11</td>
<td>33,6463</td>
</tr>
<tr>
<td>12</td>
<td>31,9197</td>
</tr>
<tr>
<td>13</td>
<td>34,9663</td>
</tr>
<tr>
<td>14</td>
<td>36,2382</td>
</tr>
<tr>
<td>15</td>
<td>36,5493</td>
</tr>
<tr>
<td>16</td>
<td>36,8577</td>
</tr>
<tr>
<td>17</td>
<td>38,3627</td>
</tr>
<tr>
<td>18</td>
<td>37,467</td>
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<tr>
<td>19</td>
<td>39,8109</td>
</tr>
<tr>
<td>20</td>
<td>40,3756</td>
</tr>
<tr>
<td>21</td>
<td>39,238</td>
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<tr>
<td>22</td>
<td>41,2082</td>
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<td>23</td>
<td>38,0665</td>
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<td>38,6567</td>
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<tr>
<td>26</td>
<td>35,9244</td>
</tr>
<tr>
<td>27</td>
<td>36,2382</td>
</tr>
<tr>
<td>28</td>
<td>36,8577</td>
</tr>
<tr>
<td>Average</td>
<td>35,3764</td>
</tr>
</tbody>
</table>

D. Noael

One of the objectives of research activities in the field of toxicology is to be able to evaluate the safety of a substance. To determine the safe limit of the concentration of a chemical begins with the toxicity test determining the highest dose without causing effects on experimental animals or No Observed Adverse Effect Level (NOAEL).

Swaen et al. (2010) ’s research states that NOAEL benzene is 3.0 mg / m3 or equivalent to 0.022 mg / kg which is permitted from the calculation of formulations as follows.10

\[
NOAEL\ benzene = \frac{3 \times 0.00013 \times 8}{0.1405} = 0.022\ mg/kg
\]

E. Safe Human Dose

\[
SHD = NOAEL \frac{W \text{ human}}{\text{Human km}}
\]

Based on the formula, the calculation of SHD obtained from the NOAEL value, the average animal Km, and the average human Km are:

F. Limits on Safe Benzene Concentration

Determining the safe limits of benzene concentration in gas stations around the area of Diponegoro University in Semarang uses the following formula (Soemirat, 2003).11

\[
C_{safe} = \frac{\text{SHD} \times W}{\delta \times BR \times t} \text{mg/m}^3
\]

To convert units of mg / m3 to ppm the following formula is used.

\[
C_{safe} = \frac{\# \text{ mg/m}^3}{(MW)} \times 24.5 \text{ ppm}
\]

Information:

- \( C_{safe} \): concentration of toxin in the air that is safe for workers (mg / m3)
- \( \text{SHD} \): Safe Human Dose (mg/kg)
- \( W \): Weight (kg)
- \( \delta \): % of substances absorbed by the lungs
- \( BR \): Human respiratory rate (m3 / hour)
- \( t \): Working time (hours)
- \( MW \): Molecular Weight
Based on the above formula, the results of calculating the safe concentration of benzene at the Around Regional Gas Station in Diponegoro University Semarang were obtained from the SHD value, average worker weight, percentage of substance absorption, average respiration rate of workers and average length of work time:

\[
C_{\text{safe}} = \frac{(0.00362)(55,67)}{(50\%)(0.59)(8)} = 0.08538 \text{ mg/m}^3
\]

\[
C_{\text{safe}} = \frac{0.08538 \times 24.45}{78.11} = 0.02672 \text{ ppm}
\]

The results of calculating safe limits in the air for upper workers can be used to predict toxins in the air in a safe work environment for workers if there is no determination of the Threshold value (William, 1985 in Tualeka, 2013)\(^{12}\), and to compare with the NAV that has Defined by various institutions both by the Ministry of Manpower and Transmigration, the National Standardization Agency, ACGIH, NIOSH and OSHA.

**Conclusion**

The results of the measurement of benzene concentrations in gas stations around the area of Diponegoro University in Semarang at point 1 were 0.38 ppm and at point 2 was 0.51 ppm. So that the concentration is still below the Threshold Value (NAB) according to the Minister of Manpower Regulation Number 5 of 2018. Based on manual calculations for safe limits benzene concentration is obtained 0.08538 mg / m3 or 0.02672 ppm.

This means that the highest concentration of benzene in the work environment is 0.51 ppm, with an average body weight of 55.67 kg, height of 159 cm with a working time of 8 hours, and safe concentration so as not to cause non-carcinogenic risk is 0.08538 mg / m3 or 0.02672 ppm.

**Conflicts of Interest:** All authors have no conflicts of interest to declare.

**Source of Funding:** The source of this research costs from self.

**Ethical Clearance:** This research was approved by the institutional Ethics Board of Airlangga Surabaya University. All subjects received complete information about the procedure and purpose of this study, each subject before the study signed an informed consent form.

**Reference**

Effect of Local Anesthesia and Extraction on Mouth Opening

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Abstract
Background: Trismus is defined as painful limitation in mouth opening due to a muscle spasm. Trismus, also called lockjaw, (restricted jaw range of motion). There are various causes of trismus, one is spasm of the muscles of mastication. Assessment of the effect of local anesthesia and extraction on mouth opening ability.
Methodology: Total sample of 40 patients included both genders (27 male and 13 female) participated in this study, all Patients were healthy with no allergy to local anesthesia, don’t use alcohol or any drug therapy. Lidocaine HCL 2% with epinephrine 1:80,000 is used to anesthetize the inferior alveolar nerve using IAN block technique, while surgical tools were used for extraction in the usual way. Baseline mouth opening was evaluated pre-operatively, during the effect of local anesthesia and post-operatively by using digital Vernier caliper. Results: significant difference in mouth opening between the measurements (pre-operative, during anesthesia, post-operative) is found, mouth opening decreases when measurement is directed toward postoperatively.

Keywords: local Anesthesia , Extraction , Mouth opening

Introduction
Trismus is defined as painful limitation in mouth opening due to a muscle spasm\[1]. Trismus, also called lockjaw, (restricted jaw range of motion). There are various causes of trismus, one is spasm of the muscles of mastication \[2]. Permanent trismus occurs less frequently than temporary trismus, in the majority of cases trismus is temporary, and typically resolves in less than two weeks \[3]. Many problems can arise as a result of restricted mouth’s opening movement, these include difficulties in eating and swallowing, oral hygiene issues, and even speaking problems. Although trismus is not prevalent in the population, it can be commonly seen in specific groups, particularly in those who have had oral surgery to extract their wisdom teeth and those who had cancer involving structures that affect mouth movement\[4]. Maximum mouth opening in the majority of people is beyond 35 millimeters (mm) wide, which is little more than the width of two fingers \[5]. There are many causes implicated in the development of trismus. One classification depends upon the involvement of the temporomandibular joint (TMJ) or intra articular versus extra articular etiologies\[6]. Others have classified the causes into broad groups such as infectious, traumatic, and neoplastic sources \[5]. As trismus can occur due to disease entity, otherwise, it may also be iatrogenic, caused by prescribed interventions and treatments\[7]. Patients suffering from trismus, appears with many manifestations such as limited ability for mouth opening and deviation of jaw to the affected side, prevalent facial swelling and fever, sensation of muscle stiffness and sever pain in acute condition \[8]. Fibrosis of the TMJ may be the result of prolonged trismus, necessitating directed therapy\[9]. Management of trismus is commonly directed toward relieving symptoms. Symptom-directed interventions such as heat therapy, analgesics and muscle relaxants may be prescribed in the acute phase of uncomplicated transient trismus. In addition, stretching exercises is another treatment protocol that may also be considered for patients with post-traumatic and post-operative trismus, particularly when persisting longer than one week. Intensive physiotherapy and microcurrent therapy may be the treatment of choice for chronic trismus especially those cases resistant to more conservative approaches. Xanthine derivatives such as pentoxifylline also prescribed for trismus\[5,9].

SUBJECTS, MATERIALS and METHODS
This study was conducted on a total sample of 40 patients included both genders (27 male and 13 female) reporting to the Department of Oral and Maxillofacial Surgery, Babylon University/ College of Dentistry for extraction of their mandibular posterior teeth. Patients were coming to college between January 2019 and March 2019 with asymptomatic bilateral mandibular posterior teeth, patients’ age was between 26 to 52 years regardless of their gender.

All Patients were healthy, with no allergy to local anesthesia, don’t use alcohol or any drug therapy that may interfere with local anesthesia. Those with poor vital signs (hypoxia, hypotension, hypertension, heart rate, respiratory rate), untreated hypertension, severe cardiac failure, glaucoma, hyperthyroidism, raised intracranial pressure, neurotic traits or psychiatric illness or those with a history of cerebrovascular accident were excluded from the sample. Pregnant or lactating mothers were also excluded.

Lidocaine HCL 2% with epinephrine 1:80,000 is the type of local anesthesia that was used. The inferior alveolar nerve was anesthetized with one carpule of local anesthesia by using IAN block technique. surgical tools like straight elevator and lower posterior teeth forceps were used for extraction in the usual way (figure 1).

Baseline mouth opening was evaluated pre-operatively, during the effect of local anesthesia and post-operatively by measuring the distance between the mesio-incisal corners of the upper and lower right central incisors at maximum opening of the jaws (Figure 3) by using digital Vernier caliper (Figure 2).
Results

Due to the use of local anesthesia, and extraction, a decrease in mouth opening was occur to all patients who participated in this study. When the mean value of all measurements (preoperative, during anesthesia, postoperative) were calculated, results show different in the measurements: where pre-op mean value is 47.07, during anesthesia is 46.05 and post-op is 45.47. As a result the scores of mouth opening decreased when patients were subjected to anesthetic agent and extraction. Regarding to ANOVA test: when we compared between Group A (pre-op mean value) and Group B (after anesthesia), we found a significant difference in their values. Same results were found when comparing Group B and Group C (post-op). Whereas a highly significant difference was found between Group A and Group C.

Table (3.1): The statistical significance of the mean value of mouth opening between the different groups assessed by ANOVA test.

<table>
<thead>
<tr>
<th>Group</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between group(A) and group(B)</td>
<td>439.160</td>
<td>13</td>
<td>33.7</td>
<td>4.092</td>
<td>0.001</td>
</tr>
<tr>
<td>Between group(B) and group(C)</td>
<td>469.781</td>
<td>13</td>
<td>36.137</td>
<td>5.130</td>
<td>0.001</td>
</tr>
<tr>
<td>Between group(A) and group(C)</td>
<td>577.310</td>
<td>13</td>
<td>44.408</td>
<td>11.088</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Fig. (3.1): Depicts how mouth opening was measured using a Vernier caliper.

Fig. (3.1): This scheme explain the significant differences between measurements of values for the groups (A,B,C).
It is observed that there is a significant difference between group A and group B values, also significant difference is found between group B and group C While highly significant difference is found between group A and group C.

Discussion

In this study, in order to know the effect of local anesthesia and extraction on mouth opening, measurement of mouth opening in three stages (pre-operative, during anesthesia, and post-operative) is necessary. After that comparison of the values is done. There are many difficulties in this study that affect the accuracy of measurements, weakness of muscles due to trauma is one of these difficulties, trauma may be due to needle breakage as it is bent or otherwise used incorrectly \(^{[10]}\). Also, forceful insertion can cause sudden movement of the patient so may harm the muscles of mastication causing weakness which affect on mouth opening \(^{[10-12]}\).

On the other hand Intramuscular injections can lead to hematoma formation and consequently fibrosis, which lead to trismus \(^{[5]}\). Hematoma is a blood-filled swelling. It can form when the injection needle strike a blood vessel \(^{[13,14]}\). After anesthesia, trismus is usually caused by intramuscular injection of the anesthetic agent into the pterygomandibular space, usually affecting either the lateral pterygoid muscle or the temporal muscle. Inflammation of these muscles usually occur and it is the result of the cytotoxic effect of the anesthetic solutions, eventually, the inflammation leads to contraction of the muscles leading to trismus \(^{[15]}\). Naturally, these cytotoxic reactions are commonly seen in block anesthesia more than in infiltration anesthesia \(^{[16]}\). Trismus can occur even 2 – 5 days after inferior alveolar block anesthesia \(^{[15]}\). The concentration and dose of local anesthesia may affect on mouth opening. When local anesthesia is injected into a highly vascular area, there is an increased risk of intravenous injection and this increases the concentration of local anesthesia in the circulation too rapidly, lastly a toxic reaction can occur. In addition, over dosage can lead to intoxication which result in an impairment in muscle movement eventually trismus results \(^{[15]}\). Kai Sundquist found that patients may experience difficulties in opening their mouth especially after the extraction of mandibular teeth and nerve block anesthesia to the lower jaw. These problems may continue for several months and are treated mainly by mouth opening exercises in order to stretch the related muscles \(^{[17]}\). Also Donna Christiano explained how trismus can arise after oral surgery, particularly after lower wisdom teeth extraction. Trismus can happen due to the inflammation the surgery creates or due to the hyperextension of the jaw during the procedure. It can also occur when a needle delivering the anesthetic solution damages the surrounding tissues \(^{[4]}\). Therefore these findings are both in an agreement with the findings of our study. It has been suggested that increased difficulty of third molar surgery may be attributed to patient factors; such as age, gender, size and ethnic background. Patient age, in particular, has been linked with increased surgical time and complications \(^{[18]}\), so increases muscular tone that result in trismus. Therefore, there was a significant difference in mouth opening between the measurements (pre-operative, during anesthesia, post-operative), mouth opening decreases when measurement is directed toward postoperatively.

Conclusion

Various studies regarding the effect of local anesthesia and extraction on mouth opening have been done over the years, based on this study, the following conclusions are drawn: when local anesthesia is administered to patients it will cause several effects, one of them is decreased mouth opening by their anesthetic effect on inferior alveolar nerve which has branch that supply mylohyoid muscle and anterior digastric muscles which assist lateral pterygoid muscle in mouth opening. Also, extraction of mandibular posterior teeth leads to decrease mouth opening due to increased muscular tone during extraction so patients will suffer from decreased muscular movement that affect on ability to open their mouth in normal width.

Ethical Clearance: The project plan displayed on the scientific committee and scientific ethical committee and get approval

Source of Funding: There is no funding source and it is completely covered by authors

Conflict of Interest: There is no conflict of interest

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Physiological and Gen Study for Animals Exposed to Leaves of Plant (Eucalyptus) from Selected Locations at, Al-Nassiriy/ Iraq

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Abstract

Our study was carried out during the period from November 2018 to May 2019. 24 rats were used assigned and divided into three groups. The first group was the control group and a second group were injected with aqueous extraction of Eucalyptus leaves extract at concentrations of 100 and 150 mg /Kg experiment lasted 30 days.

Zn, Pb, Cd, and Cu were measured in the leaves of plant and after confirming the presence of these heavy metals, we exposed the laboratory animals to the Eucalyptus aqueous extract and after the end of the period rats were sacrifice and blood collected. Blood were used to measure study parameters and relative gene expression of (SOD and catalase). Moreover, antioxidants and enzymes AST and AL were measured from serum.

Results showed that the concentration of Pb in the plant was 0.19μg/g. The highest mean Zn was 14.23μg/g. The Cd concentration was 0.35μg/g and the copper concentration were 2.31μg/g from previous data we can find out that the amount of heavy metals is not really high. we would be expected low impact on animals since not high concentration found. The results of blood parameters of rats showed different outcome where it has been noticed that a significant increase in Hb, PLT and RBC at the 100mg/kg exposure and an increase in WBC in both concentrations. In addition, our results showed a significant decrease in RBC and Hb at the 150mg/Kg decrease MDA, MCV and MCH levels are decreased at both concentrations. A significant decrease in AST at 150mg/kg while the results showed a significant increase in lower concentration.

Keywords: Eucalyptus, heavy metals, blood parameters.

Introduction

Plants are considered the oldest friends of mankind and always play a major role in the living of human and animal, and it’s important to live organisms. They are not just provided shelter and food, but they are also used in treatment of various diseases [1].

Environmental pollution has been a major area of concern worldwide. Environmental pollution, especially by heavy metals is one of the most important factors in the destruction of biosphere components[2]. Heavy metals remain in the soil for a long time and have a residence time ranging from a few to several hundred years [3]. Human exposure to heavy metals has increased due to excessive use of daily life and has increased in most parts of the world, particularly industrialized countries. Therefore, pollution from industrial activities is a major environmental problem requiring attention[4].

Heavy metals are pervasive environmental toxicants that have been shown to exert oxidative stress on living systems through the production of reactive oxygen species which overwhelm the cell’s capacity to maintain a reduced state. Metal-induced ROS cause damage to cellular proteins, nucleic acids and lipids, It has been clearly demonstrated that ROS interfere with the expression of a number of genes and signal transform pathways [5, 6].

This study aimed was measuring heavy metals in plant Eucalyptus taken from power station and the extent of the impact of the polluted plant on laboratory animals to reveal the influence of this plant on blood parameters,
liver enzyme and gene alternation.

**Method**

The plant leaves Eucalyptus samples were collected from Power station in the city of Al-Nasiriyah, Thi-Qar province, Iraq. The plant brought to the lab and washed with distilled water then dried in air. After complete dryness, the plant collected in a glass container at room temperature. Heavy metals were measured in the plant extract using a FAAS. Aqueous extract of the plant was prepared, and then laboratory rats of Rattus norvegicus species exposed to aqueous extracts of the Eucalyptus leaves that are taken from the power station at 2 concentration 100 and 150mg/kg once daily for 30 days. Blood parameters for rats were measured using hematological analyzer (Nihon Kohden). Lipid peroxidation (LPO) is determined by using the thiobarbituric acid method [7]. The activity of the enzymes alanine transaminase (ALT) and aspartate transaminase (AST) in the serum was measured through the processed kit from the company Biolabo (France). The heavy metals were determined in the liver and blood rats by using FAAS. Genomic RNA was extracted from blood isolates by using RNA Extraction (Direct-zol RNA MiniPrep Zymo RNA Purification Kit (USA) and done according to company instructions in many steps. All isolates of blood were detected by Real-Time polymerase chain reaction (RT-PCR assay) [2]

**Results**

Figure 1 showed heavy metals in Plant extract that are taken of Power Station. Lead (Pb) concentration was (0.19 µg/g) in the plant of the power station, while Zinc (Zn) have shown the higher levels in plant (14.23 µg/g). Other heavy metals are arranged according to the increase to Cu > Cd.

![Fig (1): heavy metals in Eucalyptus (µg/gm dry wt.)](image)

The results of the current study showed in Table (1) showed that the highest value of RBCs was in group 3 and lowest in group 2. As well as, showed that there is a significant increase in the level of RBC for group 2. While there was a non-significant difference in group 3 comparing to the control.

Group 3 was recorded the highest value with Hb than another group, while the lowest was group 2. As results appeared a significant rise in hemoglobin (Hb) levels in group 2 comparing to the control, as observed a non-significant difference in group 3 comparing to control group 1. The highest value of WBCs was in group 3 compared with the other group, the lowest was group 2.

Interestingly, the results of the study showed that the highest value of MCV was in group 2 and the lowest value was in group 3. Moreover, the results indicated non-significant different in MCV in the groups 2 and 3.

Likewise, the results presented the highest value of MCH was in group 2 compared with the other groups. It also exhibited results a non-significant difference in the MCH in groups 2 and 3. Group 3 was the highest value of PLT.

![Table (1): Effect of Eucalyptus extract on blood laboratory animals](table)

<table>
<thead>
<tr>
<th></th>
<th>RBCs $10^6$/mm$^3$</th>
<th>Hb g/L</th>
<th>WBCs $10^9$/L</th>
<th>MCV pg</th>
<th>MCH pg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (Control)</td>
<td>5.29 ± 0.09</td>
<td>12.03 ± 0.51</td>
<td>5.05 ± 0.28</td>
<td>66.3 ± 1.22</td>
<td>22.35 ± 0.95</td>
</tr>
<tr>
<td>Group 2 (Eucalyptus 100 mg/Kg)</td>
<td>6.17 ± 0.11</td>
<td>12.85 ± 0.20</td>
<td>9.42 ± 0.35</td>
<td>67.15 ± 1.02</td>
<td>27.18 ± 0.71</td>
</tr>
<tr>
<td>Group 3 (Eucalyptus 150 mg/Kg)</td>
<td>7.77 ± 0.42</td>
<td>15.23 ± 0.39</td>
<td>16.45 ± 0.69</td>
<td>61.25 ± 0.78</td>
<td>23.18 ± 0.91</td>
</tr>
</tbody>
</table>

♦ Values refer to mean ± SD and P value.
Group 3 was recorded the highest value with MDA than another group. MDA level a non-significant different in the groups 2 and 3 comparing to the control group1.

The present study showed that the highest value of ALT was in group 3. It also showed the highest value of AST was in group 2 table 3.

it also observed significant differences in the enzyme AST and non-significant differences in the enzyme ALT were observed in group 2. Moreover, we found that non-significant differences in group 3 in both enzymes comparing to the control group table 3.

Table (2): Effect of Eucalyptus extract on liver enzymes.

Level of mean Zn in liver of animals that drank plant extraction higher than its values in the control group. The highest values of Zn were in group 3.

Table (3): Heavy metal concentrations in liver.

The results of the present study showed that the higher values of Zn in the group 2. The results also showed non-significant differences in groups 2 and 3.

The statistical analysis shows non-significant difference in groups 2 and 3.

The present study showed that the highest values of relative expression of SOD and catalase in group 3,

Table (4): Heavy metal in rat’s blood.

Plants are considered for a long time as the most sensitive to environmental biodiversity because of their high sensitivity to the toxicity of heavy metals, and effective as the first phase in the food chain that collects pollutants compared to other organisms [3]. Over decades, plant contamination would give a clearer picture of pollution than in other measurements. [8].
The current study revealed that all the metals were accumulated to less concentrations by the plant studied in power station, except that of Zn. The outcome of the study come along with previous studies [9, 10]. According to Alloway (2013) the presence of heavy metals in the plant is due to plant growth in polluted soil containing heavy metal. exposing to extract of Eucalyptus showed that there was a significant increase in the RBC level in the group (2) of the power station. These outcomes may be due to medicinal plants which are rich in nutrients including minerals such as calcium, zinc and potassium [11]. Extract from the same plant exhibited a significant decrease in the RBC level in blood of animals that drank high concentration of these plants (150mg/kg). These interesting results would be due to the thyroid dysfunction and hormones that are produced from effect of toxins produced in the animal body after giving the animals a polluted plant extract. Animals hormones are playing a major influence in the direction of metabolism within body and have an indirect effect on the production of blood cells in the bone marrow [12].

As well as, exposing to Eucalyptus increased in the Hb levels in the group 2. According to Smith et al (2006) increased RBC cause increased hemoglobin [13]. Another logical reason to increase level of Hb may be because of a defect in the kidneys result of heavy metals resulting in an increase in hemoglobin [14]. 150mg/kg of same plant extract taken from power station influenced differently on Hb level when significant reduction noticed after 30 days’ exposure. To explain this different would be because of reactive oxygen species (ROS) that cause hemoglobin damage. It produces deposits inside the red blood cell and thus decomposes red blood cells. Likewise, it may play role in reduction of the hormone Erythropoietin and malnutrition resulting from loss of appetite causes these hemoglobin deficiency and anemia [15].

Not only that, level of WBC also influenced by Eucalyptus where level is increased. According to researchers finding, plant is content active compounds which have proven pharmaceutical effectiveness such as flavonoids, Coumarin, Glycosides and saponins [16]. These compounds are capable to activate immune system and thus be able to increase WBC. In contrast, our results showed that there are a significant decrease in levels of MCV and MCH in groups (2 and 3 ) from Eucalyptus extract. It has been proved that the reduction of MCV and MCH in blood are return to high blood lead level and low iron (because of bowel perturbation) this decrease causes a defect in the production of hemoglobin, resulting in oxygen deficiency [17].

A study done by Saidana and his colleague who reported that there is direct impact of the active compounds extracted from Suaeda plant on bone marrow of animals to generate a large number of platelets. Whereas, the reduction in PLT levels that have been noticed in the group ( 5 ) of the power station, may be due to metals ability that can
induce abnormal responses in the immune system.

Malondialdehyde (MDA) is a final product of lipid peroxidation after exposure to ROS and many studies have used it as a marker of oxidative stress evaluation [18]. In this investigation, a non-significant in the MDA levels in the Eucalyptus aqueous extracts. These results are in agreement with other studies which reported [19]. The influence of back to plant curcumin contains which decreases the oxidative stress [20]. In the present study, ALT and AST levels Eucalyptus that are collected from power station act differently where we noticed a significant increase AST level and decrease ALT in Group 4. Increasing the enzyme synthesis of liver cells to remove the toxic effect of metals. Customarily, the rise of ALT refers to liver damage and this disorder may occur in other organs that secrete this enzyme such as heart and muscles. Chavan and Muley (2014) explained that exposure to lead and cadmium increases the level of AST and do not affect ALT. for this reason, here we think the impact of lead and cadmium was clear on animals[21]. The accumulation of minerals in the blood this can probably be attributed to various factors including in general, metabolic processes, water and food contamination, and the nature of lipid concentration in tissues[22]. This variation may be due to plant extracts contaminated with heavy metals taken from the power station.

The results of the current study showed that increases in the rate of relative expression SOD and catalase. The induction of SOD and catalase expression would be because of exposure to heavy metals. Activation of SOD and catalase in response to stress signals to result from a disruption of this association, releasing SOD and catalase [23]. The current study was the first study that aimed to find the connection between contaminated plant from contaminated area that placed around the power station and animals’ health. Highly comminuted plant that are approved before by several of environmental scientists’ group around are.

**Conflict of Interest** – Nil

**Source of Funding**- Self

**Ethical Clearance** – Not required

**References**

Uncategorized References


2. 2007. 112-118.


Effect of Metronidazole on Clopidogrel Bisulphate Efficacy in Male Albino Rats

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Abstract

The data about this combination effect on platelet function and hematological parameters are sparse, but there is only mention in many medical science websites such as Medscape, there are possible interaction between it, in which metronidazole cause inhibition to cytochrome CYP3A4 which responsible on clopidogrel metabolism, this may put the patients that on clopidogrel bisulphate therapy at risk of clot formation and may be progress, this may occur because clopidogrel is at inactive form and need to activation by cytochrome mention above, so this study was performed in order to achieve the following aims: studying the efficacy of clopidogrel bisulphate on coagulation process by measuring bleeding time when giving clopidogrel alone and in combination with metronidazole and studying the possible effect of two drug alone and in combination on hematological parameters in male albino rats.

Keywords: Clopidogrel bisulphate, metronidazole, cytochrome CYP3A4, bleeding time

Introduction

Clopidogrel, a thienopyridine derivative, binds precisely also permanently to the platelet P2RY12 purinergic receptor, constraining ADP-mediated platelet motivation and aggregation (¹). Next oral taking of clopidogrel is promptly absorbed owned to more metabolism, clopidogrel is one of prodrug that is absorbed in the intestine and activated in the liver, then it converted to its active metabolite needs dual sequential oxidative steps, at the first time result in formation of 2-oxo-clopidogrel, followed by the conversion of 2-oxoclopidogrel to the active metabolite. CYP2C9 and CYP3A4/5 are connected to cytochrome P450 enzymes involved in the metabolism of clopidogrel (²). CYP2C9 is most important hepatic enzymes involved in the formation of clopidogrel’s active metabolite. Genetic polymorphisms of CYP2C9 are associated with impaired clopidogrel metabolism in healthy volunteers and also in patients (³). Metronidazole is antibiotic and antiprotozoal medication. it is one type for a first event of mild-to-moderate Clostridium difficile colitis if vancomycin or fidaxomicin is unavailable (⁴). It has been suggested possible inhibition of cytochrome P450 especially CYP2C9 and CY3A4, this enzyme is responsible for several drugs metabolism such as S-warfarin, etc.⁵. And this may appear in revers effect when combined with clopidogrel when compared with warfarin because the difference between two drugs chemically (warfarin is active drug and this combination cause abnormal bleeding).

Materials and Mthod

Experimental animals: A total number of twenty male Albino Wistar rats weighed (200-250 g) were utilized in this research. Their ages ranged between 4-5 months. Rats in all time of the experiment were housed in plastic cages in conditioned room (22-25°C) in the animal house of College of pharmacy – University Of Anbar with manually controlled lightening supplying daily light of 12 hours (7.00 AM to 19.00 PM) and 12 hours night cycle. They were left for 7 days for adaptation with the research conditions. Rats had free access to water and diet along the experimental period.

Experimental Design: Rats were randomly divided into four equal groups (5rat/group) and administered orally for 28 days by using special gavage needle as the following: C group rats that received ordinary distilled water and served as control, T1 group rats were treated
1.25 mg/kg B.W of clopidogril bisulphate, T2 group Rats were treated 35 mg/kg B.W of metronidazole and T3 group rats were treated 1.25 mg/kg B.W of clopidogril bisulphate + 35mg/kg B.W of metronidazole.

**Bleeding time test performance:** Bleeding time was measured via rat tail transection method according to the prescription of The tail of the rat was warmed for 60 seconds in water at 40°C then dried (8). A slight cut was made in the mid of the tail with a blade. Account started when the 1st drop touched the circular filter paper. It was tested at 30 s intervals till bleeding cosseted

*Total number of spot on filter paper/2= BT /minute.

**Hematological Analysis:** All animals in experiment comparing with the normal values of blood parameters as control and zero time. The blood collected from heart puncture after general anesthesia by using (Ketamine and xylazine). By using Uto-analyzer (setting on vet mode) Platelet count, RBC, WBC, and HB level were estimated. The procedure done according to manufactured instruction.

**Statistical Analysis:** The data was accomplished by using SAS (Statistical Analysis System - version 9.1). One-way ANOVA, two-way ANOVA and Least significant differences (LSD) post hoc test were complete to assess significant differences among means. P < 0.05 was considered statistically significant.

**Results**

Before beginning of dosage of experiment (at zero time), Bleeding time and other hematological parameters was determined and there was no significance different (P < 0.05) in all groups.

1. **Bleeding time:** A statistical analysis indicated that the mean value of bleeding time was non-significantly (P<0.05) differed in all experimental groups at zero time when compared to each other. The final results showed significant (P>0.05) increased after oral intubation of clopidogrel bisulphate, metronidazole and combination in T1, T2 and T3 groups comparing to the control group and zero time. The T1 group that receive 75 mg/Kg/day of clopidogrel bisulphate was more potent in increasing of bleeding time comparing to 35 mg/kg/day of metronidazole and in combination treated group (T2, T3) respectively.

<table>
<thead>
<tr>
<th>Group</th>
<th>Day of experiment</th>
<th>Zero time</th>
<th>End of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>A2.40±0.18a</td>
<td>A2.30±0.12d</td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>B2.50±0.22a</td>
<td>A13.90±0.18a</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>B2.60±0.18a</td>
<td>A3.80±0.12c</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>B2.50±0.15a</td>
<td>A7.20±0.12b</td>
<td></td>
</tr>
<tr>
<td>LSD</td>
<td>0.4831</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Different Capital letters denote differences between groups, (P<0.05).

- Different Small letters denote differences within group, (P< 0.05).

2. **Platelet count:** The effect of oral intubation of 75 mg/Kg/day of clopidogrel bisulphate (T1), 35 mg/Kg/day metronidazole (T2) and combination dose of both of them T3 for 28 day on treated rats RBCs count are clarified in table 2. There were none considerable (P<0.05) differences in platelet count between empirical groups in zero time, at the finish of research there was a significant (P<0.05) reduction in platelet count in T1 and T3 groups respectively compared with control group, zero time and T2 group. A high significant decreases (P<0.05) were recorded in animal of T1 group followed by T3 (group of combination treatment).

<table>
<thead>
<tr>
<th>Group</th>
<th>Day of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>A200.40±0.50ab</td>
</tr>
<tr>
<td>T1</td>
<td>A201.80±0.58a</td>
</tr>
<tr>
<td>T2</td>
<td>A201.20±0.86a</td>
</tr>
<tr>
<td>T3</td>
<td>A199.80±0.37b</td>
</tr>
<tr>
<td>LSD</td>
<td>1.6797</td>
</tr>
</tbody>
</table>

- Table 1: Effect of oral intubation for 28 days with Clopidogrel bisulphat, metronidazole and their combination on bleeding time (by tail method as prescribed previously) of treated adult male rats. (min)

- Table 2: Effect of oral intubation for 28 days with Clopidogrel bisulphat, metronidazole and their combination on platelet count of treated adult male rats (X10^9/µl)
- Different Capital letters denote differences between groups, (P<0.05).

- Different Small letters denote differences within group, (P<0.05).

3. Red blood cells count: A statistical analysis indicated that the mean value of RBCs count was non-significantly (P<0.05) differed in all experimental groups at zero time when compared to each other. At the end of experimental RBCs count was significantly (P>0.05) decreased after oral intubation of clopidogrel bisulphate, metronidazole and combination (clopidogrel bisulphate plus metronidazole) in T1, T2 and T3 groups comparing to the control group. The extent of decrement was higher in T1 group, where as in T3 group (combination therapy) the change was at little degree when compared with T1 group.

Table 3: Effect of oral intubation for 28 days with Clopidogrel bisulphat, metronidazole and their combination on RBC count of treated adult male rats (X10^6/µl).

<table>
<thead>
<tr>
<th>Group</th>
<th>Day of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero time</td>
</tr>
<tr>
<td>Control</td>
<td>A8.84±0.62a</td>
</tr>
<tr>
<td>T1</td>
<td>A8.33±0.09a</td>
</tr>
<tr>
<td>T2</td>
<td>A8.35±0.09a</td>
</tr>
<tr>
<td>T3</td>
<td>A8.26±0.10a</td>
</tr>
<tr>
<td>LSD</td>
<td>0.6602</td>
</tr>
</tbody>
</table>

- Different Capital letters denote differences between groups, (P<0.05).

- Different Small letters denote differences within group, (P< 0.05).

4. WBC count: Table 4 illustrates the mean value of WBC count in the control and treated group throughout the experimental period. It can be seen that WBC count were in the normal in all groups at the zero time, after animals received clopidogrel bisulphate, metronidazole and combination of them a significant fall (p<0.05) in WBC count., a significant (P<0.05) decrease in WBC count was observed in all treated groups (T1, T2, T3) comparing to the pretreated period but at a different extent more potent in T1 followed by T3 and T2 group.

Table 4: Effect of oral intubation for 28 days with Clopidogrel bisulphat, metronidazole and their combination on WBC count of treated adult male rats (X10^3/µl).

<table>
<thead>
<tr>
<th>Group</th>
<th>Day of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero time</td>
</tr>
<tr>
<td>Control</td>
<td>A9.56±0.10a</td>
</tr>
<tr>
<td>T1</td>
<td>A9.53±0.07a</td>
</tr>
<tr>
<td>T2</td>
<td>A9.55±0.12a</td>
</tr>
<tr>
<td>T3</td>
<td>A9.51±0.06a</td>
</tr>
<tr>
<td>LSD</td>
<td>0.2184</td>
</tr>
</tbody>
</table>

- Different Capital letters denote differences between groups, (P<0.05).

- Different Small letters denote differences within group, (P< 0.05).

5. Hemoglobin concentration: Table 5 illustrates the mean value for Hb concentration in the control and treated group throughout the experimental period. It can be seen that hemoglobin concentrations were in the normal in all groups at the zero time, after animals received clopidogrel bisulphate, metronidazole and combination of them a considerable fall (p<0.05) in hemoglobin concentration after 28 days of the treatment as compared with pretreated period and control group, this decrease is coincided with the decrease of RBCs in all groups of experiment.

Table 5: Effect of oral intubation for 28 days with Clopidogrel bisulphat, metronidazole and their combination on Hemoglobin concentration of treated adult male rats (mg/dl).

<table>
<thead>
<tr>
<th>Group</th>
<th>Day of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero time</td>
</tr>
<tr>
<td>Control</td>
<td>A14.76±0.15a</td>
</tr>
<tr>
<td>T1</td>
<td>A15.05±0.11a</td>
</tr>
<tr>
<td>T2</td>
<td>A14.84±0.12a</td>
</tr>
<tr>
<td>T3</td>
<td>A15.03±0.15a</td>
</tr>
<tr>
<td>LSD</td>
<td>0.3522</td>
</tr>
</tbody>
</table>
Discussion

It was reported that clopidogrel bisulphate cause a significant change in bleeding time in 36 human volunteer after giving a 75 mg of Plavix (7). Also another study indicated that clopidogrel bisulphate associated with significantly increased risk for postoperative wound complications especially increasing of bleeding time in tested patient (8). These normally cause increasing of bleeding in T1 group. In T2 group treated with metronidazole alone, it was reported a partial inhibition platelet aggregation with dose and time dependent in guinea pig and human platelet-rich plasma and this may cause a mild increasing in bleeding time according to dose that we give in comparison with previous study (9).

In T3 the combination of clopidogrel bisulphate and metronidazole which sharing same metabolic enzyme or cytochrome that may lead to inhibition or slowing the process of clopidogrel bisulphate converting to its active form (10), this mention why the combination of two drugs causing less bleeding time than clopidogrel bisulphate alone treated group. Many of case report indicates that in addition to the increased risk of bleeding with the use of Clopidogrel, some of its additional serious side effects include agranulocytosis, aplastic anemia/pancytopenia, thrombotic thrombocytopenic purpura, acquired hemophilia A, and neutropenia, neutrophils make up

Figure (1): showed the steps of experiment performance starting from housing of animals (A), dosing of the drugs (B), (C) anesthetizing animals, (D) bleeding time test, and (E&F) blood collection for hematological test parameters.
a significant portion of the WBCs (11). With regard to metronidazole there was reported that metronidazole can cause bone marrow depression (12). And normally this can affect all type of blood cells especially in chronic use.

**Conclusion**

The efficacy of clopidogrel bisulfate as blood thinner agent may be reduced when combined with metronidazole despite of the second have characteristic of antiplatelet agent, this were obvious in bleeding time test results and other hematological parameters, may be due to the inhibition of metronidazole to cytochrome that responsible for clopidogrel metabolism, in addition to possibility of compete the two drugs and/or inhibition of each other. This still need to more clearing by check the platelet activity in presence of this combination by more specific tests and apparatus. more attention should be paid when use the metronidazole in patient who take clopidogrel bisulfate especially critical cases by putting the patient under close monitoring or use alternative drugs.

**Conflict of Interest:** Personal relationship

**Source of Funding:** Self

**Ethical Clearance:** Institutional

**References**


Abstract

Polycystic ovary syndrome (PCOS) is a status that impact a woman’s hormone levels. A total of (128) samples was divided into four groups (Obese with PCOS, low weight with PCOS, Obese without PCOS, and Healthy control) collected from Kamal Al-Samarraie hospital, Ministry of Health in Baghdad-Iraq during the period of April 2017- August 2017. The aim of the study to evaluation from Serum Adiponectin Irisin and Apelin in Iraqi obese women patients with PCOS. The result shows The BMI of obese with PCOS patients and obese without PCOS was significantly higher (P<0.05) when compared to the values of the control group. No significant with other groups, also Significant increase of Prolactin (p<0.05) in Obese with PCOS and low weight with PCOS groups in relation to Obese without PCOS and control groups. A non- significant elevation (p>0.05) when comparing between Obese without PCOS, and control groups. The levels of FSH, LH and Testosterone showed significantly change (p<0.05) in Obese with PCOS and low weight with PCOS groups when comparing with Obese without PCOS and control groups. Followed by no-significant with other groups, also shows significant change (p<0.05) when comparing between Obese without PCOS and control group, showed that adiponectin level showed a significant higher level in the control group, Obese without PCOS group, low weight with PCOS patients, and Obese with PCOS patients. Oppositely, the results of the Irisin showed a significant higher level in the Obese with PCOS patients then the low weight with PCOS group followed by the Obese without PCOS and control group. While Apelin level recorded the highest level Obese with PCOS group.

Key Words: Polycystic ovary syndrome, Prolactin, FSH, LH, Testosterone, Body mass index, Adiponectin, Irisin, Apelin.

Introduction

Polycystic ovary syndrome is a popular endocrine condition related With hyperandrogenism, infertility and metabolic dysfunction. The syndrome was officially recognized in the 1930’s by Stein and Leventhal, who associated polycystic ovaries (PCO) to the clinical features of menstrual dysfunction, infertility, hirsutism and obesity (1). Androgen overabundance and insulin impedance, the two of which have powerful genetic components, underlie a great part of the clinical display. Specifically, a few studies have demonstrated that the raise androgen levels may influence adipokine produce and, increment perversiveness and cardiovascular impacts from metabolic syndrome (2). A major proportion from women with PCOS have boosted levels of LH and natural/diminished levels from FSH (3). The excess in LH is explained by an increment of the hypothalamic gonadotropin-releasing hormone (GnRH) (4). In the Granulosa cells (GCs), FSH invigorates the expression from enzymes that metabolize androstenedione to estradiol. Studies from follicular liquids and in vitro investigations of (GCs) from anovulatory PCOS ladies show that GCs, generally, remain steroidogenically dynamic with expanded aromatase movement, comparably from non-PCOS ladies. So, expanded estradiol production in PCOS is subordinate on the ovulatory condition of the patient, yet in addition on body weight (5, 6). Adiponectin is a 244-amino-acid-long polypeptide. There are four distinct areas in adiponectin with molecular weight of 30 Kilo Dalton (30KD) (7) In Female: Adiponectin, AMPK and PPARc work in show to organize ability homeostasis in the ovary and guarantee appropriate development of ovarian follicles and oocyte.
Adiponectin is involved in endometrial changes in anticipation of embryo implantation and assume a potential role in fetal upgrowth and improvement (8).

**Materials and Method**

**Samples collection**

The current study carried out at the Kamal Al-Samarraie hospital, Ministry of Health in Baghdad-Iraq during the period from April 2017- August 2017. The study was conducted on normal Weight (50) and obese (78) with polycystic ovarian syndrome, as following groups:

- **Group (1):** Includes 50 patients. The patients were obese with polycystic ovarian syndrome.

- **Group (2):** Includes 27 patients were normal weight with polycystic ovarian syndrome.

Normal, healthy individuals: For the purpose of rapprochement, 50 healthy individuals (Control) were enrolled in this study. The individuals were recruited into the following groups:

- **Group (3):** Includes 25 individuals. The individuals were obese.

- **Group (2):** Includes 25 individuals were normal weight.

All patients must be fastened overnight [10-12 hours] when they attended at the morning of 2nd-3rd day of their menstrual cycle. Five milliliters of blood was collected from each patient and control into gel tube to obtained of serum centrifuged the tube at 3000 rpm for 5 min.

**Measurements**

Enzyme-linked immunosorbent assay (ELISA) were used to estimate the serum level of Adiponectin according to the manufacturer’s instructions (bioaim scientific) and Hormonal analysis for LH, FSH, Testosterone, and prolactin was performed by using the Automated Immune Assay (AIA) by the VIDAS auto analyzer, (BioMérieux Company, France).

**Results**

The results illustrated in table (1) showed BMI within first group was 32.2 Kg/m², while the second group recorded 21.09 Kg/m², and third group were 32.8 Kg/m² compared to control group 21.12 Kg/m². The obese subjects who suffered from PCOS and obese that not suffered from PCOS recorded BMI values significantly higher (P<0.05) in comparison to control group. In addition the comparison between the BMI of low weight subjects with PCOS with control group were insignificant (P>0.05), and a non-significant differences in obese with PCOS patients as compared to obese without PCOS.

**Table (1) Comparison Body mass index between controls and PCOS patients.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. Subjects</th>
<th>Body mass index (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>50</td>
<td>32.2*a</td>
</tr>
<tr>
<td>(Obese with PCOS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td>27</td>
<td>21.09*b</td>
</tr>
<tr>
<td>(low weight with PCOS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 3</td>
<td>25</td>
<td>32.8*a</td>
</tr>
<tr>
<td>(Obese without PCOS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 4</td>
<td>25</td>
<td>21.1</td>
</tr>
<tr>
<td>Healthy control</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

♦Results are expressed as mean± SE. ♦ANOVA: Obese with PCOS and Obese without PCOS vs. Control: * p< 0.05, ♦b ANOVA: low weight with PCOS vs. Healthy control: * p< 0.05.

The results in table (2) can be summarized as follow. The prolactin level showed a significant increase (p<0.05) in Obese with PCOS and low weight with PCOS groups in contrast to Obese without PCOS and control groups. While a non-significant increment (p>0.05) appeared when comparing Obese without PCOS and control groups. The hormones FSH, LH and Testosterone levels were changed significantly (p<0.05) in both group of PCOS (Obese and low weight) when comparing with Obese without PCOS and control groups, while comparing between those groups of the PCOS obese and low weight showed non-significant change (p>0.05) while significant change (p<0.05) shown when comparing between Obese without PCOS and control group.
### Table (2): Comparison of endocrine parameters between control and PCOS patients groups.

<table>
<thead>
<tr>
<th>Parameters Groups</th>
<th>Prolactin ng/ml (mean+SD)</th>
<th>FSH mIU/ml (mean+SD)</th>
<th>Testosterone ng/ml (mean+SD)</th>
<th>LH mIU/ml (mean+SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1</strong> (Obese with PCOS)</td>
<td>A 15.066+1.945</td>
<td>A 7.617+1.095</td>
<td>A 0.638+0.072</td>
<td>A 8.274+2.165</td>
</tr>
<tr>
<td><strong>Group 2</strong> (low weight with PCOS)</td>
<td>A 14.264+1.839</td>
<td>A 7.019+1.554</td>
<td>A 0.694+0.067</td>
<td>A 8.261+2.096</td>
</tr>
<tr>
<td><strong>Group 3</strong> (Obese without PCOS)</td>
<td>B 9.628+2.074</td>
<td>B 5.496+0.957</td>
<td>B 0.955+0.064</td>
<td>B 6.253+1.255</td>
</tr>
<tr>
<td><strong>Group 4</strong> Healthy control</td>
<td>B 8.855+1.417</td>
<td>C 3.895+0.672</td>
<td>C 1.251+0.263</td>
<td>C 4.238+1.195</td>
</tr>
</tbody>
</table>

LSD 2.517 1.061 0.097 0.896

P-value 0.0115 0.0053 0.00728 0.0029

Significant Significant Significant Significant Significant

*different letters mean significant difference

The result in the table (3) showed that a significantly higher level of adiponectin in control subjects (22.461+2.350) followed by the Obese without PCOS group (19.767+2.244) then the low weight with PCOS patients (13.252+1.154) and Obese with PCOS patients (10.236+1.288). while in contrast to adiponectin, the results of the Irisin showed a significant higher level (p= 0.009) in the Obese with PCOS patients (1445.8+289.0) then the low weight with PCOS group (999.2+85.5) followed by the Obese without PCOS and control group (782.1+46.4 and 776.6+94.7, sequentially). While apillin level recorded the highest level Obese with PCOS group (11.431+0.984, p= 0.0102).

### Table (3): Levels of Adiponectin, Irisin, and Apelin in PCOS women and healthy controls.

<table>
<thead>
<tr>
<th>Parameters Groups</th>
<th>Adiponectin (ng/ml) (mean+SD)</th>
<th>Irisin (pg/ml) (mean+SD)</th>
<th>Apelin (ng/ml) (mean+SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1</strong> (Obese with PCOS)</td>
<td>C 10.236+1.288</td>
<td>A 1445.8+289.0</td>
<td>A 11.431+0.984</td>
</tr>
<tr>
<td><strong>Group 2</strong> (low weight with PCOS)</td>
<td>B 13.252+1.154</td>
<td>B 999.2+85.5</td>
<td>B 5.120+0.301</td>
</tr>
<tr>
<td><strong>Group 3</strong> (Obese without PCOS)</td>
<td>A 19.767+2.244</td>
<td>C 782.1+46.4</td>
<td>B 5.119+0.241</td>
</tr>
<tr>
<td><strong>Group 4</strong> Healthy control</td>
<td>D 22.461+2.350</td>
<td>C 776.6+94.7</td>
<td>B 3.826+0.301</td>
</tr>
</tbody>
</table>

LSD 2.06 104.27 2.11

P-value 0.016 0.0094 0.0102

Significant Significant Significant Significant

*different letters mean significant difference
Discussion

Numerous studies have proved that polycystic ovary syndrome (PCOS) have involved in obesity, as the insulin resistance increase the body mass and dyslipidemia which in turn lead to an increased chance for cardiovascular disease and diabetes (9). Numerous studies proved that that abdominal obesity increased in PCOS women of fertile age compared with healthy subjects (9,10). Obesity the last decades are considered as major chronic diseases all around the world. As previously mentioned PCOS is associated with excess weight. So for that reasons the obesity treatment should be based editing the lifestyle by diet, exercise and behavior modification. It has been reported that, that ovulation increased after losing as minimum 5 % of the initial body weight in obese women (11).

In usual, women with PCOS are usually described by increased circulating LH levels in addition decreased FSH levels (12). While some other PCOS patients with hyper-androgenism and especially to those have obesity, shown non-elevated basal or stimulated LH levels and those may lead to heterogeneity of presentations of the syndrome. The using of LH level as biomarker of GnRH so negative correlation between the levels of GnRH and LH has also been shown in studies (13,14) proved that gonadotropin releasing may increase by hypothalamus which may lead to increased release of follicle stimulating hormone (FSH) from the pituitary gland in PCOS. In addition Shibli-Rahhal and Schlechtesuggested that prolactin modulate the body weight as there is an association between prolactin level and weight gain, but still uncertain the mechanism whether obesity associated with hyper-prolactin due to stimulation of lipogenesis or due to disruption of central nervous system (CNS) (15). While, Hernandez (16) added that a decreased level of dopamine (DA) could cause the raise in prolactin level which usually present in women with PCOS. In our study, patients with PCOS had significantly higher (P<0.05) levels of testosterone compared to controls. And those results agreed with previous studies the average level of testosterone increased in the most of PCOS patients. In addition androgen action declining with aging. but also in controls, androgen levels gradually decrease through time, as has been shown in several studies (17,18). Numerous research have examined adiponectin levels in women with PCOS, and our present results agreed with who proved the lower adiponectin levels in the obese PCOS group than in the non-obese control group. The other parameter Irisin, was discovered for factors secreted by muscle in response to exercise in 2012 (19). And because of its activity in reducing body weight so still hypothesized that circulating irisin is increased in a compensatory manner to overcome or counteract the increasingly aggravated IR, and such condition can be found in metabolic disorders disease such as PCOS (20). Our results agreement with (21) who reported irisin level higher in patients with PCOS than that in the healthy controls. That could be similar to apelin level which also showed increased levels in patients and its related with the occurrence of obesity. Those results disagreed with (22) who reported lower apelin level in normal-weight PCOS women than in control subjects. In contrast, our results were in agreement with (23,24,25) who reported that the average level of apelin is higher in PCOS.

Conflict of Interest: There is no conflict of interest among the authors.

Funding: Self

Ethical Clearance: This study is ethically approved by the Institutional ethical Committee.

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Antibacterial and Antifungal Activities of
Centaureabruguierana (Asteraceae) from Iraq

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Education for Pure Sciences, TikritUniversity, Iraq, 3Department of Biology, College of Education, University of
Garmian, Iraq

Abstract

The methanol and aqueous extracts of Centaureabruguierana. (Asteraceae) from Iraq were tested for their
biological activity against two Gram positive , two Gram negative bacteria and four fungal species by using
agar well diffusion method . The crude methanol extract (100mg/ml) and its dilutions and the crude aqueous
extract( 100mg/ml) showed biological activity against all bacteria and fungi studied. Compared with the
aqueous extracts , methanol extracts, particularly the crude one, were more bioactive against bacteria and
fungi studied . Among the studied microbes, E.coli, Staphylococcus aureusand Microsporumcanis were the
most sensitive species to C.bruguierana extracts tested. Methanol extract at all concentrations and aqueous
extract at high concentrations showed close or more antibacterial activity( except in case of P.auruginosa)
than that of the bacterial control ( tetracycline) . The crude methanol extract was more bioactive against all
fungi studied than the fungal control ( nystatin ).This is the first report on the antibacterial and antifungal
activities of C. bruguierana extracts from Iraq and its bordering countries.

Kew words : Antibacterial, Antifungal , Asteraceae, Centaurea , Iraq.

Introduction

Centaurea is the fourth largest genus in the Asteraceae
family , including 300 -700 species[1][2][3][4] with annual,
biennial and perennial plants [5] that distributed all
around the world especially in Mediterranean sea region
and west Asia [1][4]. In Iraq, this genus was represented
by more than 30 species [6]. Species of this genus have
been used in folk medicine as diuretic , stomachic
, tonic, astringent, antimalarial, antirheumatic,
antidandruff, hypoglycemic and antipyretic [2][7][8][9][10].
Many investigations have been indicated the presence
of different classes of active compounds in this genus
which in many cases are responsible for its biological
activity[10][11]. C. bruguierana (DC.)Hand.-Mazz. was
reported for the first time from Iraq [12]. Our previous
study [13] showed a high flavonoid content in this species.
However, there is no reports on the antimicrobial activity
of C. bruguierana in the literature .Therefore, this study

was conducted to evaluate the effects of the methanol
and aqueous extracts of this species in four bacterial
species ( two Gram positive and two Gram negative )
and four fungal species .

Materials and Method

Plant materials and extraction method

C.bruguierana samples were collected from Tikrit
province/ Salahadin Governorate (north central Iraq )in
March 2018 . Plant samples were identified according
[14] and confirmed by the Iraqi National Herbarium .Identified
samples were kept in the Department of Biology, College
of Education for Pure Sciences,TikritUniversity,Iraq .
Aerial flowering parts of C.bruguierana were oven dried
at 40°C and then grinded with electrical mill. 40 g of
the powdered samples were extracted with 160ml of
95% methanol or hot distilled water for 24 hours, then
the liquid was filtered through gauze colth and filter
paper Whatman No.1 and the filtrate was concentrated
at 40°C. The solid residue was stored in the fridge until
subsequent use . For in vitro assay ,1g of the solid
residue were dissolved in 10ml of the same solvents
(methanol or distilled water) and tested at concentrations 25,50,100mg/ml. These concentrations were evaluated in triplicate for bacteria or fungi tested. Before use, the crude extracts (100mg/ml) were filtered by using Millipore filter with Whatman filter paper No. 0.22 [15][16].

Microorganisms and antimicrobial assay

Two Gram positive(Staphylococcus aureus, Bacillus pumilus) and two Gram negative( Escherichia coli, Pseudomonas aeruginosa) bacterial species, three fungal species(Microsporum canis, Trichophyton rubrum, Aspergillus terreus) and one yeast (Candida albicans) were used in this study. All bacteria and molds were obtained from the Biology Department, College of Science, Baghdad University and the yeast was supplied from the Department of Biology, College of Science, Tikrit University. Nutritive media used included nutrient agar and MacConkey agar for bacteria and Sabouraud dextrose agar and potato dextrose agar for fungi(all media from Bekasi, Indonesia). Antibacterial and antifungal activities were studied by agar well diffusion method [16][17]. For the antibacterial and antifungal tests, tetracycline and nystatin (100µg/ml for each) were used as positive control while methanol and sterilized distilled water were used as negative control.

Results and Discussion

The biological activities of the methanol and aqueous extracts of C.bruguierana against bacterial and fungal species tested in this study are presented in Table 1; Fig.s.1,2. The results showed that all C. bruguierana extracts exhibited inhibitory activity against all bacterial species studied while these extracts showed some activity on fungi tested. Methanol extracts revealed strong activity against both bacterial and fungal species studied compared with the aqueous extracts. The activity of the studied extracts was directly proportional with their concentrations. As shown in Table 1, S. aureus, E. coli and M. canis were the most sensitive to the extracts tested especially at high concentrations. In case of bacteria(Table 1 and Fig.1), methanol extract of C.bruguierana at all concentrations showed inhibition zone diameters (16.1-25mm) very close or greater than the standard antibiotic tetracycline (16.4mm). Methanol extract only at high concentration (100mg/ml) gave inhibition zones diameter(17-20mm) greater than the antifungal nystatin(15mm). Aqueous extracts except few cases (B.pumilus and P.aeruginosa) showed inhibition zones close or greater (15.1-25.4 mm) than the control tetracycline (Table 1 and Fig.2). Aqueous extracts at all concentrations showed lower inhibition zones diameter than the antifungal nystatin. Table 1 revealed that the mean inhibition zone diameters exhibited by the methanol extracts(19.1-23.6mm for bacteria and 14.2-16.7mm for fungi) were greater than those exhibited by the aqueous extracts (3.0-22.6mm). The high flavonoid contents previously reported in C. bruguierana [13] may be responsible for the antimicrobial activity of this species [10][11]. According to the literatures, this is the first report on the antimicrobial activity of the C. bruguierana. The present study was in agreement with the previous studies on the antimicrobial activity of the other Centaurea species particularly their methanol extracts [3][6][7][8][10][18][19]. However, further studies are needed to evaluate the biological activity of methanol extracts of C. bruguierana against other pathogenic bacteria and fungi because their antimicrobial activities are more than that of the of the standard antibiotic tetracycline and antifungal nystatin.

Table 1. Antibacterial and antifungal activities of the methanolextract from C.bruguierana

<table>
<thead>
<tr>
<th>Microbes</th>
<th>methanol extract mg/ml</th>
<th>aqueous extract mg/ml</th>
<th>mean methanol extract</th>
<th>mean aqueous extract</th>
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<tbody>
<tr>
<td></td>
<td>25</td>
<td>50</td>
<td>100</td>
<td>25</td>
</tr>
<tr>
<td>S. aureus</td>
<td>20.9</td>
<td>24.9</td>
<td>25.0</td>
<td>20.0</td>
</tr>
<tr>
<td>B. pumilus</td>
<td>16.1</td>
<td>19.2</td>
<td>22.6</td>
<td>13.2</td>
</tr>
</tbody>
</table>
Continued... Table 1. Antibacterial and antifungal activities of the methanolextract from C. bruguierana

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>E. coli</th>
<th>P. aeruginosa</th>
<th>C. albicans</th>
<th>T. rubrum</th>
<th>M. canis</th>
<th>A. terreus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>020.</td>
<td>23.2</td>
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<td>M. canis</td>
<td>14.0</td>
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<tr>
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<table>
<thead>
<tr>
<th>Antibiotics</th>
<th></th>
</tr>
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<tbody>
<tr>
<td>tetracycline</td>
<td>16.4</td>
</tr>
<tr>
<td>nystatin</td>
<td>15</td>
</tr>
<tr>
<td>Methanol</td>
<td>0.0</td>
</tr>
<tr>
<td>water</td>
<td>0.0</td>
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</table>

![P. aeruginosa](image1.png) ![C. albicans](image2.png) ![T. rubrum](image3.png)

![M. canis](image4.png) ![A. terreus](image5.png)

**Fig. 1.** Antimicrobial activity of the methanol extracts of *C. bruguierana*.
St: standard
Conclusion

This is the first study on the antimicrobial activities of C. bruguierana extracts. This study showed a promising antimicrobial activities of C. bruguierana extracts, particularly methanol extracts. Methanol extracts exhibited stronger antibacterial and antifungal activities than the standard antibiotic tetracycline and the antifungal nystatin. This plant species could be a source for antimicrobial drug against pathogenic microorganisms, particularly multiresistant bacteria.

Conflict of Interest: There is no conflict of interest among the authors.

Funding: Self

Ethical Clearance: This study is ethically approved by the Institutional ethical Committee.

References


Effects of Laparoscopic Ovarian Drilling on Restoration of Menstrual Cycles, Hormonal Profiles and Pregnancy Rate in Women with Polycystic Ovary Syndrome

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Abstract

One of the major cause of an ovulatory infertility is polycystic ovary syndrome with a multifactorial etiology. Laparoscopic ovarian drilling (LOD) is regarded as the method of choice for inducing ovulation in those not responding to oral ovulation induction with PCOS.

A prospective study was carried out for assessment of effects of LOD on menstruation, serum levels of hormones, and reproductive outcome including ovulation and pregnancy rate.

A total of 169 patients with an ovulatory infertility underwent LOD over a period of about two years. All patients assessed three times for clinical characteristics and serum hormone levels LH, FSH, testosterone, SHBG, prolactin and AMH. FAI and LH/FSH ratio calculated, first before the procedure, second within one week after and the last one after three months with the ovulation and pregnancy outcome follow up until six month.

The prevalence of irregular menstrual pattern was 89.3%, significantly (P< 0.001) decreased to 19.5% after the procedure with improvement of hirsutism, acne and scalp hair loss, there was a highly significant decrease in the serum levels of LH, testosterone, AMH, FAI and LH/FSH ratio (P ˂ 0.001) and a highly significant elevation of serum SHBG and prolactin post-operatively, resumption of spontaneous ovulation of 56.2%, and the pregnancy rate of all the study group was 26%. In conclusion, LOD regarded as a method for improvement of the hormones, restoration of menstruation and increasing ovulation and pregnancy rate.

Keywords: PCOS: Polycystic ovary syndrome; LOD: Laparoscopic ovarian drilling,

Introduction

PCOS is defined as one of the most common female endocrinopathy affecting reproductive aged women around the world and a major cause of anovulatory infertility¹. Has been defined using various criteria including menstrual irregularity, hyperandrogenism, and polycystic ovary morphology². Until recently no universally accepted clinical definition existed for this condition³. The prevalence of PCOS, like that of any other complex multifactorial disorder, depends on which criteria are used for definition. Affecting 6-21%of reproductive aged women, depending on population studied and diagnostic criteria applied⁴. Gonadotrophin abnormalities in PCOS include LH hypersecretion due to increased LH pulse amplitude in 30-90% of women with PCOS⁵. FSH levels tend to be normal or reduced compared to regularly ovulating women, resulting in an increased LH/FSH ratio⁶. Laparoscopic ovarian drilling was first described by Gjonnaess in 1984⁷ as an alternative to ovarian wedge resection to treat PCOS, to destroy the tissues that produce androgen and decrease conversion of androgens to estrogens peripherally. Specifically, serum levels of androgens and LH fall and level of FSH have been demonstrated to increase after LOD⁸. These changes in hormones thought to convert the adverse androgen dominant intra follicular environment to estrogenic and
normal ovarian pituitary feedback mechanism. The aim of this study was to define the effectiveness of LOD on menstrual cycle regulation, normalization of the hormones after the surgery and the rate of ovulation and pregnancy in an ovulatory infertility PCOS patients who failed to respond to medical treatment.

Patients and Method

This was a prospective, cross sectional study carried out on 169 women confirmed to have PCOS based on Rotterdam criteria (at least two of the following criteria were present: oligo-anovulation, clinical or biochemical hyperandrogenism, and polycystic ovaries on ultrasonography who were clomiphene-resistant (failed to ovulate after maximum dose of clomiphene for 3-6 cycles), allocated to undergo LOD, attending outpatient fertility clinic in Maternity Teaching Hospital, public maternity hospital in Erbil, Iraq. Patients were recruited from September, 2016 to November, 2018. All women enrolled were informed about the study with informed consent and they were subjected to detailed history and examination.

The age of the included subjects was between 20-39 years with no male or tubal factor infertility. Those with other etiologies of hyperandrogenism, AMH<4 ng/ml and FSH >12.5 mIU/ml were excluded from the study.

Blood analyses were conducted three times first at the second to fourth day of the cycle without ovulation induction before, second within one week and the third one three months after the procedure for serum hormones including LH, FSH, total testosterone, SHBG, prolactin and AMH. Serum hormone levels were measured by Elecsys machine (Roche Diagnostics, Hitachi, Switzerland) which was used for determination of human LH, FSH, total testosterone, prolactin and SHBG levels. AMH was measured by Enzyme linked immunosorbent assay (ELISA) kit. LOD performed for both ovaries by or in the presence of the first author, using mono polar diathermy at 40 watts making 4 punctures in both ovaries for 4 seconds each, with follow up for ovulation and pregnancy rate up to 6 months

Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS, version 22). Numerical variables were summarized as means and standard deviations. Categorical variables were presented in form of frequencies and percentages. Paired t test was used to compare the means before and after laparoscopy. McNemar test was used to compare the percentages before and after the laparoscopy. A p value of < 0.05 was considered statistically significant.

Results

The study included 169 infertile women with polycystic ovary syndrome (PCOS) not responding to medical treatment under went LOD we didn’t observe any intraoperative or postoperative complications. The mean age±SD of the women was 29.63±4.5 years, ranging from 20 to 39 years, and the median was 29 years as presented in Table 1 which shows that the largest proportion (45.6%) of the sample lies in the age group 25-29 years. The duration of infertility of 65.7% of the sample was five or more years. The majority of the women(76.9%) had primary infertility. Regarding the menstrual pattern, it was irregular in 89.3% of the sample (mostly as oligomenorrhea) and more than half (59.2%) of the women were obese.

It is evident in Table 2 that the prevalence of the medical conditions mentioned in the table had significantly decreased after LOD except for acanthosis nigricans (p=0.238). It is worth to mention that the prevalence of irregular menstrual pattern was 89.3% before laparoscopy, which significantly(p<0.001) decreased after laparoscopy to 19.5%.

Table 3 shows that the means of the following hormone had significantly (p<0.001) increased after one week of laparoscopy: LH, FSH, SHBG and prolactin, while there was a significant decrease in the means of the following: testosterone(p<0.001), AMH(p<0.001), FAI(p<0.001), and LH/FSH ratio (p=0.005). The same pattern can be observed after three months except for the LH where it is evident that its mean decreased to 7.17(p<0.001).Table 4 shows a significant decrease in the means of the following hormones three months after laparoscopy compared with the means of one week after laparoscopy: LH(p<0.001), testosterone (p=0.001), AMH(p<0.001), FAI (p<0.001), and LH/FSH ratio(p<0.001), while there was significant increase in the following means: FSH (p=0.001), SHBG(p<0.001), and prolactin (p=0.029).Finally, results showed that the ovulation rate was 56.2%, and the total pregnancy rate within six months was 26%.
Table 1. Basic characteristics of the study sample.

<table>
<thead>
<tr>
<th></th>
<th>No. (%)</th>
<th>Mean (+ SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-24</td>
<td>19 (11.2)</td>
<td></td>
</tr>
<tr>
<td>25-29</td>
<td>77 (45.6)</td>
<td></td>
</tr>
<tr>
<td>30-34</td>
<td>42 (24.9)</td>
<td></td>
</tr>
<tr>
<td>35-39</td>
<td>31 (18.3)</td>
<td>29.63 (+4.5)</td>
</tr>
<tr>
<td>Duration of infertility (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 5</td>
<td>58 (34.3)</td>
<td></td>
</tr>
<tr>
<td>≥ 5</td>
<td>111 (65.7)</td>
<td>5.6 (+2.28)</td>
</tr>
<tr>
<td>Type of infertility</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>130 (76.9)</td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>39 (23.1)</td>
<td></td>
</tr>
<tr>
<td>Menstrual pattern</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regular</td>
<td>18 (10.7)</td>
<td></td>
</tr>
<tr>
<td>Irregular</td>
<td>151 (89.3)</td>
<td></td>
</tr>
<tr>
<td>Pattern of irregular menstruation (n = 151)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oligomenorrhea</td>
<td>125 (74.0)</td>
<td></td>
</tr>
<tr>
<td>Amenorrhea</td>
<td>26 (15.4)</td>
<td></td>
</tr>
<tr>
<td>BMI (Kg / m²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 25</td>
<td>17 (10.1)</td>
<td></td>
</tr>
<tr>
<td>25-29</td>
<td>52 (30.8)</td>
<td></td>
</tr>
<tr>
<td>≥ 30</td>
<td>100 (59.2)</td>
<td>30.02 (+4.00)</td>
</tr>
<tr>
<td>Total</td>
<td>169 (100.0)</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Prevalence of some clinical features before and after the LOD

<table>
<thead>
<tr>
<th>Medical conditions</th>
<th>Before N = 169</th>
<th>After N = 169</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irregular menstrual pattern</td>
<td>No. (89.3)</td>
<td>No. (19.5)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Hirsute</td>
<td>126 (74.6)</td>
<td>97 (57.4)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Acne</td>
<td>86 (50.9)</td>
<td>21 (12.4)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Acanthosis negricans</td>
<td>36 (21.3)</td>
<td>30 (17.8)</td>
<td>0.238</td>
</tr>
<tr>
<td>Greasy skin</td>
<td>116 (68.6)</td>
<td>27 (16.0)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Scalp hair loss</td>
<td>70 (41.4)</td>
<td>49 (29.0)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 3. Means of the hormones, before, one week, and three months after LOD

<table>
<thead>
<tr>
<th></th>
<th>Before Mean (+SD)</th>
<th>A week after Mean (+SD)</th>
<th>P*</th>
<th>Three months after Mean (+SD)</th>
<th>P**</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH</td>
<td>10.13 (+3.24)</td>
<td>11.44 (+3.20)</td>
<td>&lt; 0.001</td>
<td>7.17 (+1.85)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>FSH</td>
<td>4.58 (+1.13)</td>
<td>5.53 (+1.05)</td>
<td>&lt; 0.001</td>
<td>5.88 (+1.25)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>T</td>
<td>0.85 (+0.53)</td>
<td>0.65 (+0.37)</td>
<td>&lt; 0.001</td>
<td>0.46 (+0.30)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>SHBG</td>
<td>20.25 (+8.74)</td>
<td>28.50 (+13.76)</td>
<td>&lt; 0.001</td>
<td>38.09 (+17.01)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>AMH</td>
<td>7.45 (+1.74)</td>
<td>5.89 (+1.42)</td>
<td>&lt; 0.001</td>
<td>4.75 (+1.23)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>FAI</td>
<td>16.50 (+11.53)</td>
<td>8.94 (+5.89)</td>
<td>&lt; 0.001</td>
<td>4.93 (+4.20)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Prolactin</td>
<td>19.98 (+9.55)</td>
<td>30.14 (+32.42)</td>
<td>&lt; 0.001</td>
<td>35.06 (+22.33)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LH/FSH</td>
<td>2.29 (+0.78)</td>
<td>2.12 (+0.67)</td>
<td>0.005</td>
<td>1.27 (+0.41)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

*Comparison between the means before and one week after laparoscopy.

** Comparison between the means before and three months after laparoscopy.
Table 4. Means of the hormones, one week, and three months after LOD

<table>
<thead>
<tr>
<th></th>
<th>A week after</th>
<th>Three months after</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (+SD)</td>
<td>Mean (+SD)</td>
<td></td>
</tr>
<tr>
<td>LH</td>
<td>11.44 (+3.20)</td>
<td>7.17 (+1.85)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>FSH</td>
<td>5.53 (+1.05)</td>
<td>5.88 (+1.25)</td>
<td>0.001</td>
</tr>
<tr>
<td>T</td>
<td>0.65 (+0.37)</td>
<td>0.46 (+0.30)</td>
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</tr>
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<tr>
<td>Prolactin</td>
<td>30.14 (+32.42)</td>
<td>35.06 (+22.33)</td>
<td>0.029</td>
</tr>
<tr>
<td>LH/FSH ratio</td>
<td>2.12 (+0.67)</td>
<td>1.27 (+0.41)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Discussion

LOD regarded as one of the mode of PCOS treatment and this surgical treatment renovated by assumption of different surgical technique with use of different energy modalities to increase the success rate and minimize the complications\(^{11}\).

In the current study menstrual cycle after LOD reported improvement from 89.3% to 19.5% this is in agreement with result of Seyam et al\(^{12}\) and other studies\(^{13,14}\). Also hair suite, has a significant change and improvement in our study these results are in agreement with the study of Ashrafinia et al \(^{13}\). In another study prevalence of hair suite from 60% after intervention become 40%. Also acne improvement from 50.9% to 12.4% these findings similar to results in many other previous studies \(^{13,15}\), although many authors in their studies with laparoscopic surgery not reporting on this outcome and other clinical characteristics like acanthosis nigricans, greasy skin and scalp hair loss in our study\(^{2}=0.238, p<0.001, p=0.001\) respectively, more studies required to know about them after this modality of treatment.

Improvement in the endocrine data in our work is a remarkable observation are comparable with those previously reported showing a significant decrease in LH, testosterone, LH/FSH ratio\(^{11,16,17}\), significant increase of LH\(^{p<0.001}\) few days post operatively in our study confirming report of the same phenomena by\(^{16}\), this finding is against those found in previous studies\(^{9,18}\)showed no change in the level of LH and testosterone weeks and months after operation. Significant increase in SHBG in the current work confirmed by study \(^{19}\), furthermore another study demonstrated no change in this hormone\(^9\). The obtained result in the present study confirmed previous report\(^{17,20}\) of significant reduction of FAI.

Elevation of FSH is observed in the current and different studies\(^{16,17}\), secondary increase in FSH is controversial, studies reported no change\(^{11,13,21}\) although one study done by Elgafor demonstrated reduced FSH in his results\(^{22}\) this variability in the results may be due to different age groups, sample size and technique in their study. Hormones produced by ovaries immediately after intervention restores feedback to the hypothalamus and pituitary lead to increased pituitary sensitivity and temporarily higher LH and particularly FSH levels.
Folliculogenesis initiation is induced by the increased FSH levels, in combination with decrease number of follicle and reduced intra-ovarian androgen levels\(^\text{23}\).

As mentioned by Farzadi et al risk of ovarian tissue damage may not be significant statistically and decrease in AMH to the level of normality without any effects on the ovarian reserve\(^\text{18}\). In our study decrease in AMH to a significant level but within normal range from mean of (7.45 before to 4.75 after the drilling) may be due to the significant increase in FSH and this may affect the negative feedback of the AMH secretion and also in the present study the mean AMH pretreatment was higher compared to the mean of AMH in\(^\text{18}\).

The ovulation and pregnancy rate in the present study were the two main outcome measures (56.2\% and 26\%) respectively near to the results observed in the study\(^\text{12}\), were (60\% and 27.5\%) respectively, these results in agreement with other studies\(^\text{15,18}\). In several other studies described ovulation and pregnancy success results ranging from 26.5\%-94\%\(^\text{9,11,24}\) and 20\%-84\%\(^\text{9,16,25}\) respectively. In the present study despite of 56.2\% ovulation rate but the conception rate was not high during the six months follow up compared to the most other studies may be related to the duration of follow up after the operation reaching up to 12-18 months and this explain the long term effects of LOD, number of punctures used in each study and the last may be due to hyper prolactinemia observed in our study in some of the patients and this is in accordance to the previous finding\(^\text{26}\) while another study observed no change in this hormone\(^\text{17}\), so follow up of prolactin level after ovarian surgery is important.

Conclusions

Thus LOD not only helps in regulating ovulation and enhancing conception rates but also helps much in regulation of menstruation and hormone level improvement for future steps of infertility management for those failed to conceive a period after LOD.

Conflicts of Interest: No conflicts of interest

Ethical Clearance: The Research Ethics Committee of HMU, College of Medicine, approved the study proposal.

Source of Funding: Not

References


Association between Breast Feeding And Breast Cancer: A Case Control Study in Erbil

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Abstract

Background and Objective: Breast cancer is the most common cancer that threatens life of women all over the world. In Iraq it is the most common cancer; 5141 new cases of breast cancer registered in 2018 comprising 36.7% of all women cancers and 20.3% of all cancers in both sexes. Breast cancer is a multifactorial disease many of the risk factors are modifiable, one of these factors is breastfeeding.

The aim of the study is investigating the association of duration of breastfeeding with breast cancer development, and comparing the risk in premenopausal and postmenopausal women.

Patients and methods: This study is a prospective case control study conducted in Erbil Breast Clinic and Erbil teaching hospital in Erbil city from January 31, 2016 to June 31, 2016.

140 women with histopathologically proved breast cancer compared with 160 women with no breast cancer in regard to total duration of breastfeeding.

Results: Among the 140 women with breast cancer; 66.4% women were pre-menopausal, and 33.6% women were postmenopausal, among the 160 women in the control group; 73.1% women were pre-menopausal, and 26.9% were postmenopausal.

Among postmenopausal women; 19.15% of women with breast cancer never practiced breastfeeding, while only 2.33% of women in the control group never practiced breastfeeding which was statistically different.

Conclusion: Postmenopausal women who did not practice breastfeeding and parous women who breastfed their children less than 12 months are at increased risk of breast cancer.

Key words: women, breast cancer, breastfeeding, risk.

Introduction

Breast cancer is the most commonly occurring cancer in women and the second most common cancer after lung cancer overall, there were over 2 million new cases all over the world in 2018 ¹. In Iraq it is the most common cancer; 5141 new cases of breast cancer registered in 2018 comprising 36.7% of all women cancers and 20.3% of all cancers in both sexes².

The incidence of breast cancer greatly varies among different populations, up to a 10-fold difference in incidence have been observed between low rates in rural African and Asian populations and high rates in North American and Western European populations ³. This great variation in the incidence is related to the risk factors which may contribute to the development of breast cancer; genetic factors contribute only to 5-10% of cases, the rest 80-85% of breast cancer causes are related to behavior and endocrine risk factors, which vary according to the culture, education and income of these populations, most of these risk factors are modifiable and avoidable⁴, therefore preventing or modifying these factors can prevent or lessens the development of the disease, one of these modifiable endocrine risk factors is breastfeeding. Breastfeeding not only reduces breast cancer risk but also provides other health benefits to...
the mother including reduced risk for endometrial and ovarian cancers5, and reduced risk for chronic medical diseases that are also risk factors for cancer, such as hypertension and diabetes6.

Controversies exist in the researches about the protective role of breastfeeding against the risk of breast cancer development in the mother as general and in pre and postmenopausal women.

The aim of the study is to assess the risk of breast cancer development in pre and postmenopausal women who never breastfed and parous women who practiced breastfeeding.

**Patient and Methods**

A prospective case control study conducted in Erbil Breast Clinic and Erbil teaching hospital in Erbil city, Iraq from January 31, 2016 to July 15, 2017. Erbil Breast Clinic is the only governamental clinic specialized for management of breast problems, it daily receives 50-70 women with breast problems, it provides necessary investigations.

After taking informed consent, physical examination and necessary investigations performed for both breast cancer ad control women.

152 breast cancer women enrolled in the study, 13 women were excluded from the study because they declined to continue with the study and only 139 women with proved breast cancer continued with the study, 175 control women attending to the surgical consultation unit in Erbil teaching hospital for non-breast complaints enrolled in the study, one of these women incidentally found to have breast cancer and included in the breast cancer group, 14 other women declined from the study, and 160 women continued with the study.

The study is a part of an ongoing PhD research was approved by the ethical committee of the college of medicine/ Hawler Medical University.

The recorded information analyzed by SPSS software version 22 using student t test and Chi square test.

**Results**

This study compared 140 histopathologically proved breast cancer women with 160 control women, Among the 140 women with breast cancer; the women age range from 25 to 91 years with the mean of 46.06 years age, 93 women were pre-menopausal (66.43%), and 47 women were postmenopausal (33.57%), among the 160 women in the control group; the women age range from 25 to 70 years with the mean of 42.76 years, 117 women were pre-menopausal(73.1%), and 43 were postmenopausal (26.9%), with no significant statistical difference between the two groups: tables 1, 2 and 3.

**Table-1: Age distribution of breast cancer and control women**

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>160</td>
<td>25.00</td>
<td>70.00</td>
<td>42.76</td>
<td>10.14</td>
<td>0.390</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>140</td>
<td>25.00</td>
<td>91.00</td>
<td>46.06</td>
<td>11.24</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2: Age distribution of pre-menopausal women**

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>breast cancer</td>
<td>93(66.4%)</td>
<td>25.00</td>
<td>53.00</td>
<td>40.9±6.78</td>
<td>0.491</td>
</tr>
<tr>
<td>Control</td>
<td>117(73.1%)</td>
<td>25.00</td>
<td>51.00</td>
<td>38.24±7.10</td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>211</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3: Age distribution of post-menopausal women

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>breast cancer</td>
<td>47(33.6%)</td>
<td>46.00</td>
<td>91.00</td>
<td>57.87±8.50</td>
<td>0.329</td>
</tr>
<tr>
<td>Control</td>
<td>43(26.9%)</td>
<td>42.00</td>
<td>70.00</td>
<td>55.07±6.19</td>
<td></td>
</tr>
<tr>
<td>total</td>
<td>90</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4 compares the practice of breastfeeding whether never or ever breastfed, regarding the premenopausal women; 25 women with breast cancer and 21 women from the control side never used their breasts for feeding which was not statistically significant different. While more postmenopausal women in the control group had breastfed their children, which is statistically significant.

Table 4: Breastfeeding practice of breast cancer and control women

<table>
<thead>
<tr>
<th>Breast cancer women</th>
<th>Control women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breastfeeding</td>
<td>Breastfeeding</td>
</tr>
<tr>
<td>p-value</td>
<td>p-value</td>
</tr>
<tr>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Pre-menopausal</td>
<td>68(73.12%)</td>
</tr>
<tr>
<td>postmenopausal</td>
<td>38(80.85%)</td>
</tr>
<tr>
<td>Total</td>
<td>106</td>
</tr>
</tbody>
</table>

In regard to the minimum duration of breastfeeding which may protect against breast cancer; the study show that at least 12 months of breastfeeding may protect against breast cancer only in postmenopausal women Table 5 and 6.

Table 5: Total breastfeeding duration of premenopausal breast cancer and control women

<table>
<thead>
<tr>
<th>Breastfeeding practice</th>
<th>Total breastfeeding (months)</th>
<th>Breast cancer No.</th>
<th>%</th>
<th>Control women No.</th>
<th>%</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breastfeeding practice</td>
<td>Never practiced</td>
<td>25</td>
<td>26.88</td>
<td>21</td>
<td>17.95</td>
<td>0.133</td>
</tr>
<tr>
<td></td>
<td>Ever practiced</td>
<td>68</td>
<td>73.12</td>
<td>96</td>
<td>82.05</td>
<td></td>
</tr>
<tr>
<td>Women practiced breast feeding</td>
<td>1-11</td>
<td>4</td>
<td>5.88</td>
<td>9</td>
<td>9.38</td>
<td>0.561</td>
</tr>
<tr>
<td></td>
<td>12+</td>
<td>64</td>
<td>94.12</td>
<td>87</td>
<td>90.62</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1-23</td>
<td>11</td>
<td>16.18</td>
<td>18</td>
<td>18.75</td>
<td>0.836</td>
</tr>
<tr>
<td></td>
<td>24+</td>
<td>57</td>
<td>83.82</td>
<td>78</td>
<td>81.25</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>68</td>
<td>96</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 6: Total breastfeeding duration of postmenopausal breast cancer and control women

<table>
<thead>
<tr>
<th>Breastfeeding practice</th>
<th>Total breastfeeding (months)</th>
<th>Breast cancer No.</th>
<th>Breast cancer %</th>
<th>Control women No.</th>
<th>Control women %</th>
<th>p- value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breastfeeding practice</td>
<td>Never practiced</td>
<td>9</td>
<td>19.15</td>
<td>1</td>
<td>2.33</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>Ever practiced</td>
<td>38</td>
<td>80.85</td>
<td>42</td>
<td>97.67</td>
<td></td>
</tr>
<tr>
<td>Women practiced breast feeding</td>
<td>1-11</td>
<td>4</td>
<td>10.53</td>
<td>0</td>
<td>0</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>12+</td>
<td>34</td>
<td>89.47</td>
<td>42</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1-23</td>
<td>5</td>
<td>13.16</td>
<td>2</td>
<td>4.76</td>
<td>0.248</td>
</tr>
<tr>
<td></td>
<td>24+</td>
<td>33</td>
<td>86.84</td>
<td>40</td>
<td>95.24</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>38</td>
<td>42</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

Breast cancer is a multifactorial disease with many risk factors, some of them are modifiable, breastfeeding is one of the important modifiable risk factor which may have protective role against breast cancer, though there is a large debate about its role especially when related to the menopausal state.

This study concluded that breastfeeding has protective role against breast cancer in postmenopausal women; postmenopausal women who ever breastfed their babies are less susceptible to breast cancer compared with women who never practiced breastfeeding, more ever among parous women who practiced breastfeeding; at least 12 months of total duration of breastfeeding needed to have a protective role against breast cancer development but this protective role was only found for postmenopausal women, while no significant difference found in susceptibility for breast cancer among premenopausal women who never breastfed and those who breastfed less than 12 months.

A number of studies reported the protective effect of breastfeeding against breast cancer only for premenopausal women. A number of studies reported the protective effect of breastfeeding against breast cancer only for premenopausal women. Many review articles and meta-analytic articles support the protective role of breast cancer in both pre and postmenopausal women:

- A meta-analysis on four cohort studies concluded that breastfeeding protects against breast cancer, a 2% reduction in breast cancer risk for each 5 months increase of lifetime breastfeeding. The longer women breastfeed, the more they are protected against breast cancer. The 4th edition of the European Code against Cancer in 2014 recommends: “Breastfeeding reduces the mother’s cancer risk. If you can, breastfeed your baby.”

- Research by the international Collaborative Group on Hormonal Factors in Breast Cancer – based on individual data from over 50,000 women with breast cancer in 30 different countries – has shown that breastfeeding has a protective effect estimated at a 4.3% risk reduction for every 12 months of cumulative breastfeeding (in addition to an estimated 7.0% reduction in risk for each birth. The same study showed that breast cancer risk was reduced in both...
premenopausal and postmenopausal women, with no significant difference in the effect estimated according to menopausal status 27.

The hypotheses which may explain the protective effect of breast-feeding against breast cancer are:

1. Breastfeeding induces long-term endogenous hormonal changes; increased prolactin production and decreased estrogen level, which may decrease a woman’s total exposure to estrogen, thereby preventing the carcinogenesis or growth of breast cancer cells 28.

2. Most lactating women will be amenorrhic as long as they lactate, so less exposure to the cyclical elevated estrogens which enhance mitotic activity of the mammary cells during the luteal phase of the menstrual cycle 29.

3. The estrogen levels in breast fluid of lactating premenopausal women is lower compared with nulliparous women and parous women who did not practice breast-feeding30.

4. Pregnancy and subsequent breastfeeding induces differentiation of the mammary gland lobules from lobule 1 of nulliparous women to fully differentiated lobule type 4 during breastfeeding which is more resistant to carcinogenesis 29, 31.

6. Breastfeeding milk excretes some potential chemical carcinogens, hence provides temporary protection of the mammary tissue from these carcinogens 32.

7. During breastfeeding Oxytocin hormone increases which inhibits proliferation and growth of tumor cells 33.

8. Breastfeeding may decrease cancer risk by excreting DNA damaged cells 34.

**Conclusion**

Breastfeeding for at least 12 months is protective against breast cancer in postmenopausal women. It is worthwhile to encourage women to breastfeed their children to increase the protection against breast cancer.

**Conflict of Interest:** Not

**Ethical Clearance:** The study research was approved by the ethical committee of the college of medicine/ Hawler Medical University

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**Source of Funding:** Not

**References**


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33. Cassoni P, Sapino A, Papotti M, et al. Oxytocin and oxytocinanalogue F314 inhibit cell proliferation...

Anthropometric Study of Nose Parameters in Iraqi Arabic and Kurdish Subjects (Possible Personal Identification Tool)

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Abstract

Background and Purpose: The human nose has been reported to differ in its anatomy and morphology between different racial and ethnic groups. Nasal anthropometric features when studied can provide useful information for reconstruction or esthetic surgical procedures and personal identification for forensic purposes. Method: A total of 311 subjects with age (19-23) years were divided according to gender, race (Arabic or Kurdish), and residence regions in Iraq (north, middle and south). Different nose parameters were measured for the subjects using digital Vernier caliper. Results: Comparing races, the nose height and nose length were significant between Arab and Kurds, nasal index showed (60, 60.6) for Arabic vs. Kurdish, respectively. Sexual dimorphism was significant in all the parameters obtained. The fine nose was the commonest among all subjects. Parameters analysis revealed high positive significant correlation among various parameters except the nasal index that showed negative correlation with nasal height and length. Conclusions: Nasal morphometric parameters were unique for each person and have variations between different race (Arabic and Kurdish), gender variation and the correlation among various parameters been recorded for Iraqi people. These morphometric evaluations are the key for standardization among Iraqis, and in comparison with different countries, this have great values in anatomical, surgical (aesthetic) and forensic aspects regarding personal identification.

Keywords: anthropology, nasal dimension, nasal index, personal identification, Rhinoplasty

Introduction

Morphometric variations are noticed in facial features among different races [1]. The human nose has been reported to differ in its anatomy and morphology between different racial and ethnic groups [2]. Measurements of different parameters of nose became very worthy in procedures related to nasal surgery and cosmetology [3]. Nasal anthropometric features when studied can provide useful information to the rhinoplasty surgeon especially when nasal reconstruction is performed for individuals who desire to change their nasal morphology [3, 4]. The nasal shape differs in human populations like various facial structures. For example, the interval between nasal wings had higher significant values in Asian and African when compared to European race [5]. The nasal index is one of the morphometric variables among individuals. It defined as the ratio of nasal width divided to nasal height multiplied by 100. It was revealed that the nasal index was the best index for distinguishing the different human ethnicities [6, 7].

Material and Method

In this present study, a total of 311 pharmacy students were recruited for the study, having age 18-22years, at Al-Rafidain University College, Baghdad and approved by ethical committee in the university. All the subjects were normal healthy residents of Iraq, 156 male and 155 female, the study was conducted during the period from September 2018 to February 2019. The study purposes were explained to all subjects and a written informed consent was obtained from each subject. Medical history and clinical examination were obtained, none of those enrolled for the study have history of craniofacial trauma, nose diseases, congenital anomalies or surgery of the nose. Subjects are classified according to race into Kurdish race and Arabic race. In addition, subjects are also classified into their different origin; from North, middle and south of Iraq. Measurements of parameters were obtained directly from the nose by a single investigator (to eliminate error), by using a digital Vernier’s caliper. Measurements were recorded in
millimeter, to the nearest 0.1mm. Each subject measured twice for accuracy and to each dimension.

Anthropometric measurements were performed depending on the following anatomical landmarks [8,9]: nasion (n), it is the midline point nasofrontal suture and the nasal root; subnasale (sn), it is the point just at middle of columella base; alar curvature (ac), the most lateral point that could be seen at curved base line of each ala ; and pronasale (prn), the most protruding point at the nasal tip. The Anthropometric parameters that were measured include the following, and are illustrated in Figure 1:

1. Nose height (NH): distance from subnasale to nasion
2. Nose length (NL): from pronasale to nasion
3. Width of nose (WN): span from right curve of nasal alae to left one.
4. Right nostril diameter (RND) where maximum diameter of right nostril measured
5. Left nostril diameter where (LND) maximum diameter measurement was done.
6. Right alar curve pronasale (RACP) distance from right curve of ala to nasal tip
7. Left alar curve pronasale (LACP) distance from left curve of nasal ala to nasal tip
8. Nasal index was measured by calculating the nasal width divided to nasal height multiplied by 100.

Nose shape had been classified into five types according to nasal indices [10]: 1. Very fine nose with nasal index <54.9 (Hyperleptorrhine), 2. Fine nose with nasal index =55.0-69.9 (Leptorrhine), 3. Medium nose when nasal index had a value between 70.0-84.9 (Mesorrhine), 4. broad nose with nasal index = 85.0-99.9 (Platyrrhine) and 5. very broad nose when nasal index >100 (Hyperplatyrrhine).

The collected data was statistically analyzed using SPSS software v.20. Values were expressed as mean ± standard deviation (SD). T test, one way ANOVA followed by post hoc test, chi square Fisher Exact and Pearson correlation test were used to compare the differences of parameters with significance value P<0.05.

**Results**

All different nasal parameters were recorded in Table 1. The parameters were recorded as mean ± SD, comparing the two races Arabic and Kurdish in one hand and comparing males and females on other hand. Sexual dimorphism was noted with male having higher values than their female counterparts, which were significant in all obtained parameters and nasal index (Table 1).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Race</th>
<th>Mean ± SD</th>
<th>Gender</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nose height (NH)</td>
<td>Arabic</td>
<td>54.4 ± 5.9 *</td>
<td>Female</td>
<td>51.3 ± 3.8 *</td>
</tr>
<tr>
<td></td>
<td>Kurdish</td>
<td>52.6 ± 5.0</td>
<td>Male</td>
<td>56.3 ± 6.1</td>
</tr>
<tr>
<td>Nose length (NL)</td>
<td>Arabic</td>
<td>48.1 ± 6.2 *</td>
<td>Female</td>
<td>45.2 ± 4.3 *</td>
</tr>
<tr>
<td></td>
<td>Kurdish</td>
<td>46.4 ± 5.0</td>
<td>Male</td>
<td>49.8 ± 6.3</td>
</tr>
<tr>
<td>Width of Nose (WN)</td>
<td>Arabic</td>
<td>32.5 ± 4.5</td>
<td>Female</td>
<td>30.2 ± 2.9 *</td>
</tr>
<tr>
<td></td>
<td>Kurdish</td>
<td>31.7 ± 3.2</td>
<td>Male</td>
<td>34.2 ± 4.2</td>
</tr>
<tr>
<td>Right nostril diameter (RND)</td>
<td>Arabic</td>
<td>21 ± 3.2</td>
<td>Female</td>
<td>19.3 ± 2.4 *</td>
</tr>
<tr>
<td></td>
<td>Kurdish</td>
<td>20.4 ± 2.9</td>
<td>Male</td>
<td>22.3 ± 3.0</td>
</tr>
<tr>
<td>Left nostril diameter (LND)</td>
<td>Arabic</td>
<td>20.2 ± 3.6 *</td>
<td>Female</td>
<td>18.6 ± 2.5 *</td>
</tr>
<tr>
<td></td>
<td>Kurdish</td>
<td>20 ± 2.8</td>
<td>Male</td>
<td>21.7 ± 3.4</td>
</tr>
<tr>
<td>RACP</td>
<td>Arabic</td>
<td>33.5 ± 5.2</td>
<td>Female</td>
<td>30.8 ± 3.0 *</td>
</tr>
<tr>
<td></td>
<td>Kurdish</td>
<td>32.6 ± 3.9</td>
<td>Male</td>
<td>35.5 ± 5.1</td>
</tr>
<tr>
<td>LACP</td>
<td>Arabic</td>
<td>33.5 ± 4.7 *</td>
<td>Female</td>
<td>30.8 ± 3.0 *</td>
</tr>
<tr>
<td></td>
<td>Kurdish</td>
<td>32.6 ± 3.5</td>
<td>Male</td>
<td>35.6 ± 4.1</td>
</tr>
<tr>
<td>Nasal Index (NI)</td>
<td>Arabic</td>
<td>60 ± 7.9</td>
<td>Female</td>
<td>59.2 ± 6.4 *</td>
</tr>
<tr>
<td></td>
<td>Kurdish</td>
<td>60.6 ± 7.2</td>
<td>Male</td>
<td>61.2 ± 8.7</td>
</tr>
</tbody>
</table>
Comparison of anthropometric nasal measurements between the two races, Arabic and Kurdish Iraqis, a statistically significant difference was found for only four parameters; nose height, nose length, LACP and LND. On the other hand, nasal index shows no significance between the two races (Table 1). Furthermore, the correlations of different parameters among Arabic, Kurdish and total subjects were analyzed and presented in Table 2.

### Table 2: Pearson correlation for different parameters of nose among Arabic, Kurdish and total subjects.

<table>
<thead>
<tr>
<th>Correlations</th>
<th>Nasal Height</th>
<th>Nasal Length</th>
<th>Nasal Width</th>
<th>Right Nostril Diameter</th>
<th>Left Nostril Diameter</th>
<th>RACP</th>
<th>LACP</th>
<th>Nasal Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal Height</td>
<td>total</td>
<td>1</td>
<td>0.8</td>
<td>0.4</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Arabic</td>
<td>1</td>
<td>0.9</td>
<td>0.4</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>Kurdish</td>
<td>1</td>
<td>0.7</td>
<td>0.3</td>
<td>0.2</td>
<td>0.3</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Nasal Length</td>
<td>total</td>
<td>0.8</td>
<td>1</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Arabic</td>
<td>0.9</td>
<td>1</td>
<td>0.3</td>
<td>0.4</td>
<td>0.4</td>
<td>0.5</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>Kurdish</td>
<td>0.7</td>
<td>1</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Nasal Width</td>
<td>total</td>
<td>0.4</td>
<td>0.3</td>
<td>1</td>
<td>0.7</td>
<td>0.8</td>
<td>0.5</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>Arabic</td>
<td>0.4</td>
<td>0.3</td>
<td>1</td>
<td>0.8</td>
<td>0.8</td>
<td>0.5</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>Kurdish</td>
<td>0.3</td>
<td>0.3</td>
<td>1</td>
<td>0.7</td>
<td>0.7</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Right Nostril Diameter</td>
<td>total</td>
<td>0.3</td>
<td>0.3</td>
<td>0.7</td>
<td>1</td>
<td>0.9</td>
<td>0.6</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>Arabic</td>
<td>0.3</td>
<td>0.3</td>
<td>0.8</td>
<td>1</td>
<td>0.9</td>
<td>0.6</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>Kurdish</td>
<td>0.2</td>
<td>0.3</td>
<td>0.7</td>
<td>1</td>
<td>0.8</td>
<td>0.7</td>
<td>0.5</td>
</tr>
<tr>
<td>Left Nostril Diameter</td>
<td>total</td>
<td>0.4</td>
<td>0.4</td>
<td>0.8</td>
<td>0.9</td>
<td>1</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Arabic</td>
<td>0.4</td>
<td>0.4</td>
<td>0.8</td>
<td>0.9</td>
<td>1</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Kurdish</td>
<td>0.3</td>
<td>0.4</td>
<td>0.7</td>
<td>0.8</td>
<td>1</td>
<td>0.8</td>
<td>0.6</td>
</tr>
<tr>
<td>RACP</td>
<td>total</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.6</td>
<td>0.7</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>Arabic</td>
<td>0.5</td>
<td>0.4</td>
<td>0.5</td>
<td>0.6</td>
<td>0.7</td>
<td>1</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Kurdish</td>
<td>0.5</td>
<td>0.5</td>
<td>0.6</td>
<td>0.7</td>
<td>0.8</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>LACP</td>
<td>total</td>
<td>0.5</td>
<td>0.5</td>
<td>0.7</td>
<td>0.7</td>
<td>0.8</td>
<td>0.7</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Arabic</td>
<td>0.6</td>
<td>0.5</td>
<td>0.7</td>
<td>0.7</td>
<td>0.8</td>
<td>0.8</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Kurdish</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>0.5</td>
<td>0.6</td>
<td>0.6</td>
<td>1</td>
</tr>
<tr>
<td>Nasal Index</td>
<td>total</td>
<td>-0.4</td>
<td>-0.3</td>
<td>0.7</td>
<td>0.5</td>
<td>0.4</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>Arabic</td>
<td>-0.4</td>
<td>-0.3</td>
<td>0.7</td>
<td>0.5</td>
<td>0.5</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Kurdish</td>
<td>-0.6</td>
<td>-0.3</td>
<td>0.6</td>
<td>0.4</td>
<td>0.3</td>
<td>0.1</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Pearson correlation of various parameters revealed positive correlation among many parameters. The highest score was 0.9 and it noticed between Right and Left Nostril Diameter. In contrast, negative correlations were seen in nasal index with nasal height (-0.4, -0.4 and -0.6) for total, Arabic and Kurdish respectively. Another negative correlation was observed between nasal index and nasal length with same value for total, Arabic and Kurdish (-0.3) (Table 2). Nose shapes were calculated based on the number and percentage of nose shape by race (Arabic and Kurdish) and presented in Table 3. The most common shape in both races was the fine nose (68%) while the least frequency was the broad type (1%). No significant difference was observed between races and shape, indicating that the race does not have any effect on nose shape.

Table 3: Distribution of nose shape in different races, Arab and Kurd (Chi square)

<table>
<thead>
<tr>
<th>Shape</th>
<th>Arab</th>
<th>Kurd</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very fine nose</td>
<td>52</td>
<td>23</td>
<td>75 (24%)</td>
</tr>
<tr>
<td>Fine nose</td>
<td>130</td>
<td>82</td>
<td>212 (68%)</td>
</tr>
<tr>
<td>Medium nose</td>
<td>17</td>
<td>5</td>
<td>22 (7%)</td>
</tr>
<tr>
<td>Broad nose</td>
<td>1</td>
<td>1</td>
<td>2 (1%)</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>111</td>
<td>311</td>
</tr>
</tbody>
</table>

The results of the anthropometric analysis obtained among different regions of Iraq (north, middle, and south) are shown in Table 4. The middle region shows the highest parameters of all regions. One-way ANOVA test was performed to compare parameters among the three regions. A significant difference was seen only in nose height and nose length. Tukey post hoc test revealed the significance was only between the middle and north group and in both nose height and nose length (Table 4).

Table 4: Nose morphometry among different regions, north, middle and south (measurements in mm). The results were expressed as mean ±SD. One way ANOVA followed by post hoc Tukey analysis were used to compare the differences between groups. a, b and c represent significantly difference as compared to South, middle and north (P<0.05) respectively

<table>
<thead>
<tr>
<th>Region</th>
<th>Parameter</th>
<th>South N=93</th>
<th>Middle N=107</th>
<th>North N=111</th>
<th>Total N=311</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Nose height</td>
<td>54 ± 5.3</td>
<td>54.7 ± 6.4c</td>
<td>52.6 ± 5.0b</td>
<td>53.8 ± 5.7</td>
<td></td>
</tr>
<tr>
<td>Nose length</td>
<td>47.6 ± 5.4</td>
<td>48.5 ± 6.8c</td>
<td>46.4 ± 5.0b</td>
<td>47.5 ± 5.8</td>
<td></td>
</tr>
<tr>
<td>Width of nose</td>
<td>32.2 ± 4.2</td>
<td>32.7 ± 4.8</td>
<td>31.7 ± 3.2</td>
<td>32.2 ± 4.1</td>
<td></td>
</tr>
<tr>
<td>Right nostril diameter</td>
<td>20.8 ± 3.1</td>
<td>21.1 ± 3.2</td>
<td>20.4 ± 2.9</td>
<td>20.7 ± 3.1</td>
<td></td>
</tr>
<tr>
<td>Left nostril diameter</td>
<td>20 ± 3.6</td>
<td>20.4 ± 3.6</td>
<td>20 ± 2.8</td>
<td>20.2 ± 3.4</td>
<td></td>
</tr>
<tr>
<td>RACP</td>
<td>33.3 ± 4.4</td>
<td>33.7 ± 5.738</td>
<td>32.6 ± 3.9</td>
<td>33.2 ± 4.8</td>
<td></td>
</tr>
<tr>
<td>LACP</td>
<td>32.9 ± 4.6</td>
<td>33.9 ± 4.8</td>
<td>32.6 ± 3.5</td>
<td>33.2 ± 4.3</td>
<td></td>
</tr>
<tr>
<td>Nasal Index</td>
<td>59.9 ± 6.9</td>
<td>60 ± 8.7</td>
<td>60.6 ± 7.2</td>
<td>60.2 ± 7.7</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

The nasal morphometry is one of the significant features that characterize different races because it is at the center of the face. Different anthropological findings have described the nose as a signature indicating the ethnicity, race, age, and sex of an individual [11,12]. Morphometric assessments of parameters have clinical importance for nasal reconstruction after injury or for esthetic causes in addition to its importance in personal identification. Arabs had different race from Kurdish people that might help us to evaluate different race. Moreover there is variation in climate between north, middle and south of Iraq. According to our knowledge, the study of nose morphometry among Iraqis is the first study that carried out among our population, including the racial difference between Arabic and Kurdish. As presented above significant differences were noticed regarding nasal length and nasal height in both races. The dominant shape among subjects was the fine nose in both races. In addition, differences were noticed among nasal length, nasal height and nasal index. Some of the distinctive parameters that were carried out in this study are RACP and LACP. These two parameters assess distance of nasal tip and the nasal curve that provide indirect way to appreciate nasal deviation or not and the shorter distance means Ipsilateral nasal tip deviation. RND and LND parameters obtained above revealed RND and LND showed the high positive correlation only with NW and should be taken in consideration in reconstruction surgery.

Comparing our results with other results obtained from different studies, for instance among Nigerian populations [13, 14], there were lower values of nasal height and nasal length (44.1±3.7mm and 39.8±2.8mm) respectively, compared to our findings (53.8±5.7mm and 47.5±5.8 mm). However, nasal width and nasal index showed higher values (37.7±2mm and 86.1±8mm) compared to ours (32.2±4.1mm and 60.2±7.7mm) respectively. Both Nigerian studies showed similar findings to ours concerning sexual dimorphism, specifically there was significant difference in both gender regarding nasal height, nasal width and nasal index. Although, our results showed lower values of nasal width and nasal index compared to their results and higher values of nasal height. Conversely, on a recent Nigerian study, it stated lower values for nasal height, nasal length and nasal width compared to our results. Furthermore, it represented higher nasal indices for female and male (92.9, 98.9) respectively, and therefore broad nose is the dominant type, in contrast to ours, where the nasal indices for females and males were (59.2, 61.2) respectively, and fine nose is most common type. On the other hand, among Italian subjects nasal height, nasal length, nasal width and nasal index were 57.6±4mm, 49.3±4.8mm, 37.1±2.5mm and 64.9±6.9 respectively for adult male, while for females the same parameters were 54.3±4.2mm, 46.3±4.0mm, 35.3±2.6mm and 65.3±6.5 respectively [15]. Our findings showed lower values for nasal height, nasal width and nasal index, with close values for nasal length.

Conclusions

Nasal morphometric parameters had different values for each parameter in different countries and had variations in regions within same country. Our results between Arabic and Kurdish, gender variation and the correlation among various parameters were recorded for Iraqi people subsequently these morphometric evaluations are the key for standardization among Iraqis, which have important impact for anthropologists, forensic and surgical procedures, by depending on values obtained from same community. In general, our study and many studies showed different nose parameters that emphasize the effects of race, sexual dimorphism, genetic and climate conditions on parameters.

Source of Funding- Self

Ethical Clearance – Not required

Conflict of Interest: None

References


The Comparison of Social Support Against the Life Quality of the Spinal Cord Injury Under Stress

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Abstract

Introduction: The stress of war has caused severe problems for the individual and family. Spouses of veterans are gradually involved in family erosion. Lack of social protection and social dysfunction decreased life satisfaction and reduce intimacy between partners.

Purpose: This study has been done in the purpose of comparing social support against the veteran’s life who are under stress.

Method: This cross-sectional study was done on 79 posttraumatic stress disorder veteran wives and it was also done on 79 ordinary wives through convenience sampling. The instrument to collect the required information was the quality of life questionnaire which contained 36 SF questions and Medical Outcomes Study Social Support Survey (MOS-SS).and the needed information was collected by self-reported method.

Results: Veteran wives social support with the mean and the standard deviation was 19.3±5.1 while it was 31.1±3.7 versus the ordinary wives and this difference was significant (P less than 0.01). It showed the veteran wives quality of life with the mean and standard deviation of 46.3±.088 while it showed 66.9±8.5 the mean and standard deviation for the ordinary wives and it showed the significant difference (P less than 0.01).

Conclusions: the obtained result showed that the quality of life related to the health and social support in veteran wives is lower in compare with the ordinary wives with the same situation, therefore educational, treatment and supporting and promoting plans for this group is necessary.

Key word: social support; life quality; veterans; PTSD

Introduction

Trauma has many devastating effects on the patient1, 2). These effects include spinal cord injury. Social support, PTSD veteran wives, life quality. Posttraumatic stress disorder is a serious reaction and perpetual response which appears because of being exposed to serious stresses3). Posttraumatic stress disorder was identified and recognized during the civil war in the United States4). The prevalence of this disorder in the public population is 4-8 percent5). But this percentage is between 9 to 25 percent among the war injured ones during the first two years6). The prevalence of that among the Vietnamese war soldiers has been reported as much as 30.1% to 14.7%7). It has also been reported 17.1% to 15.6% for the solders coming back from Iraq war8, 9). According to the present documents more of the Iranian veterans have posttraumatic stress disorder and this disorder which is always with the veterans has a very bad effect on they themselves, their lives and their family lives10, 11). Living with a person who suffers PTSD can transfer some of the symptoms to their wives12).
so one of the war victims is the veteran wives who are exposed to the PTSD so the veteran wives encounter lots of problems in their lives and these problems become more and more by passing the time and cause them to have mental disorders and this continual disorder can bring trauma to the wives and can cause anxiety, depression, isolation in the veteran wives. The veteran wives will have family erosion little by little and because of the lack of social support they lose their satisfaction with the family members especially with their husbands. One of the anxiety of the family suffering from PTSD is mental relationship, matrimony compatibility and sexuality satisfaction which can affect their life quality. Most PTSD veteran wives sometimes face challenges that force them to be compatible to life because of their children and loving their husbands and some wives who cannot tolerate this hard situation decide to divorce. Studies have shown that most veteran wives have lack of social communications and they cannot express themselves well and they hesitate whether to continue that life or separate their husbands and in most cases the presence of their children cause them not to do so and this causes them to accept the problems and become compatible. An eight-year war with Iraq and its follow-ups have made many Iranian warfare Died and veterans suffer from war-related Mental disorders. In addition to medical and Mental disorders, this disorder decreases the sufferers’ quality of life. So, this study that has been based on the purpose of comparing the social support and life quality of PSTD veteran wives has been done.

Material and Method

This cross-sectional study was done on two wife groups; the first group were the veteran wives and this group contained 158 PSTD veteran wives and the second group consists of 79 ordinary wives. This study was done in the year 2016. The study setting was Aliabad city, the population of the city is 150,000 people and according to the announcement of The Foundation of Martyrs and Veteran Affair, there are 96 PSTD veterans there. The method of sampling was done by census method among the ones who had inclusion criteria which was certified by a psychologist, and those samples were the veterans with documents that proved they are veterans, and not addicted to any drug and abuse substances were chosen. All participants provided informed consent after the study purpose was explained, and assurances were made of anonymity and privacy. At most, 79 people were qualified for the study. The control group was elected among the veteran wife relatives and their close friends and they had no PTSD disorder and no chronic mental disorder rand they were not addicted to any drugs.

This study information was collected by Demographic questionnaire, Medical Outcome Study Social and supportive Survey (MOS-SS).

Demographic questionnaire: This questionnaire contains information such as age, education, number of children, the length of time living with his wife, drug using, and the chronic mental illnesses.

Short form of quality of life (QOL-SF36): This questionnaire which contains 36 questions measures and evaluates the physical, mental and eight domains of life. The physical life quality consists of physical function, limitation in having a role because of the physical lack in efficiency, physical pain and general health.; and domain of psychological, life quality, contains limitation in role because of having emotional problems, social function, happiness and vividness and healthy mental. The method of giving score to them was between zero for the worst to 100 according to the questionnaire instruction SF36, and the score obtained by the samples is between 0 and 100 and the reliability of this study has strongly been confirmed and the reliability of the native form of this questionnaire by Shahgholian(2014) in all aspects has firmly been confirmed and is 0.95 – 0.91.

Medical Outcomes Study Social Supports Survey (MOS-SS): This questionnaire was made by Sherbourne and Stewart. These supportive social tools have 19 questions and 5 sub scales. These subscales are tangible support (4 questions), which measures the financial and behavioral, exciting support (4 questions) which evaluates the positive emotion, sympathy, encouragement to express the feelings. Informing (4 questions) which evaluates guidance, informing, kindness (3 questions) which evaluate the amount of love, and positive social compatibility (4 questions) which evaluates the people who do the amusement and fun activities. This scale is a self-report tool which is used to measure the amount of agreement or disagreement of someone to any of them in five-point Likert scale (never = 1 point, seldom= 2 points, sometimes = 3 points, often = 4 points, always = 5 points). The lowest score in this is 19 and the highest score is 95. The high point in this test shows that the test is supported by the society a lot. The reliability of sub domain of this test was reported by Cronbach’s alpha 0.91. All statistical procedures were performed with
SPSS 16 statistical software. Using descriptive statistics independent t-test.

**Findings**

The mean age of the PTSD veteran wives were 39 individuals (49%), high school and guidance school education was 24 individuals (31%) primary school education 13 individuals (6%) and three of them had university degree, among the ordinary people wife, 48 (61%) had high school and guidance school education, (31%), 5 individuals were uneducated, 5 individuals (6%) had diploma and two of them (3%) had university degree.

The amount of life quality of the veteran wives with means and standard deviation was 46.3±0.88 (P<0.01). There also was seen a significant difference between the veteran wives versus the ordinary people (Table 1).

The amount of veteran wife’s social support by mean and standard deviation was 19.3 ± 5.1 and in the ordinary people it was 31.1 ± 3.7 (P<0.1).

<table>
<thead>
<tr>
<th>Table (1): comparing the PTSD veteran wife’s life quality</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dimension of the group</strong></td>
</tr>
<tr>
<td>----------------------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Physical dimension</td>
</tr>
<tr>
<td>Mental dimension</td>
</tr>
<tr>
<td>Life quality general score</td>
</tr>
</tbody>
</table>

**Discussion**

Spinal cord injury affects the mental health of patients, with a prevalence of mild depression of 22.6% in the Hatefi et al(24).The result of the study shows that the PTSD veteran wives have a lower quality of life. In both the physical and mental dimensions, the veteran wives had lower quality of life in compare with the ordinary people wives. Studies have shown that the veteran wives have more excitement and it has caused them to have a lower quality of life. The results of the present study were same as Bares (2011), Brow (2010) and in all these studies the PTSD veteran wife’s life quality was low(7, 25).

Clinical experiences have shown that the veteran family, particularly their wives, suffer mental problems such as seclusion feeling, loneliness, inability, sin feeling, and depression(26). because the PTSD veteran wives are exposed to stresses and have lots of turmoil in their marriage lives(13). Lots of studies show that the PTSD veteran wives suffer a lot from mental disorder in compare with the ordinary people or the wives of the veteran who have no stress(14, 27, 28). shows the surveys of this toll in the form of depression, anxiety and mental disorder and also as the physical problems and it is known as the prevalent headache and physical complaint among the veteran wives,27 because living for a long time with someone who suffers post-traumatic stress disorder has a deep and serious influence on their family and can be a chronic stress factor for their wives and can be the source of big and serious problems their families(29).

This study showed that the social support for the veteran wives is lower than ordinary people wives. Communication disorder, physical problems and its consequence, lack of understanding the veterans by some people in the society is one of the most important things that the veterans suffer from(13). Social supports may have a very important effect on developing care health and also on mental pathology in reaction to the stressful life events(30). Social supports from the authorities and the social organizations can increase respect, can reduce blame and label and it can also form a better self-compatibility in veteran wives and it also can reduce stress in their lives(14).
Conclusion

According to the findings of this study, it can be concluded that the martyr and veteran families are special families with special stresses and the amount of their stress increases day by day. Women are the center of these stresses, and they themselves and their children are at the risk of danger therefore the society is at danger. Therefore, in order to reduce the amount of stress and the factors that causes stress and anxiety in this group, presenting more and more suitable services and economical is an evitable necessity. Besides, in order to reduce the damage of stresses, teaching educational services, that is teaching life skills are among the communicative skills and stress management and doing actions on beliefs and their attitude towards the incidents and life meaning will be useful. It is also suggested that the veteran wife’s life compatibility is to be investigated through a quantitative research.

Conflict of Interest: There is no conflict of interest between authors.

Acknowledgment: This article is the result of Research Project No. 94413 Approved by the Young Researchers Club, Islamic Azad University of Aliabad Katoul. We appreciate the cooperation of the Foundation of Martyrs and Veterans Affairs and the participants in conducting this research.

Source of Funding: This work was supported by the Young Researchers Club, Islamic Azad University of Aliabad Katoul.

Ethical Clearance: Ethical considerations in this study included obtaining written consent from participants, assuring them of the confidentiality of their data, and allowing them to freely participate in this research.

References


Burning Mouth Syndrome and Salivary Brain Derived Neurotrophic Factor Level in Type 2 Diabetes Mellitus Patients on Metformin Monotherapy and on Combination of Metformin and Glibenclamide (A Comparative Study)

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1M.Sc.student, 2Assistant professor, Department of Oral Diagnosis, College of Dentistry, University of Baghdad

Abstract

Background: Diabetes mellitus is a chronic, progressive, incompletely understood metabolic condition chiefly characterized by hyperglycemia. Impaired insulin secretion, resistance to tissue actions of insulin, or a combination of both are thought to be the commonest reasons contributing to the pathophysiology of type 2 diabetes mellitus.

Objectives: The objectives of this study were to estimate the salivary Brain Derived Neurotrophic Factor level in type 2 diabetes mellitus patients on treatment of metformin as a monotherapy and those on combination treatment of metformin and glibenclamide. Also to evaluate the burning mouth syndrome in patients with diabetes mellitus under hypoglycemic agents.

Method: In this study 80 male subjects divided into 3 groups: Group 1: 30 subjects as healthy control group, Group 2: 25 subjects with type 2 diabetes under treatment of metformin as a monotherapy 500 mg twice/day and duration 12- 18 months, and Group 3: 25 subjects with type 2 diabetes treated with combination of metformin 500 mg twice/day and glibenclamide 5mg twice/day and duration 12- 18 months. Oral examination was done for each participant and the oral manifestations were recorded. Unstimulated whole saliva samples were collected to measure the brain derived neurotrophic factor level and comparing the results with each group. Body mass index for all participants was recorded. Salivary Brain derived neurotrophic factor concentrations were measured by enzyme linked immunosorbent assay ELIZA.

Results: The results showed that the burning mouth syndrome was seen in both patients groups. Statistical analysis showed a significant increase in burning mouth syndrome among the study groups. Regarding salivary brain derived neurotrophic factor, results revealed that there was a significant higher in combination group as compared to control and metformin monotherapy group.

Conclusion: Both monotherapy and combination therapy was affect salivary level of brain derived neurotrophic factor. Burning mouth syndrome was seen secondarily to diabetes and poor glycemic control and seen in both patients groups.

Keywords: Burning mouth syndrome, metformin, glibenclamide, salivary brain derived neurotrophic factor, Diabetes Mellitus.

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Low levels of insulin to achieve adequate response and/or insulin resistance of target tissues, mainly skeletal...
muscles, adipose tissue, and to a lesser extent, liver, at the level of insulin receptors, signal transduction system, and/or effector enzymes or genes are responsible for these metabolic abnormalities. Type 2 diabetes mellitus (T2DM) is a complex condition and serious health problem worldwide. In net terms, T2DM is a group of metabolic diseases characterized by chronic hyperglycemia followed by the abnormal secretion and actions of insulin. Genetic and environmental factors are thought to be responsible for the development of T2DM. Besides these, it has been noticed that T2DM is associated with inflammation. Brain-derived neurotrophic factor (BDNF) is a neurotrophin (NT) that plays an important role in maturation, synaptic connection, neuronal repair, and plasticity of the central nervous system (CNS); also it has an influence on the pathology and the treatment of neurological diseases. Besides the fundamental impact on the nervous system, several reports documented an association between plasma BDNF and systemic or peripheral inflammatory conditions, such as acute coronary syndrome and T2DM. One medication that has proved effective in treatment of type 2 diabetes and is considered the first choice for oral management, as recommended by the American Diabetes Association, is a drug in the biguanide class, metformin. Most side effects of metformin are mild and can include gastrointestinal distress, soft stools, and diarrhea. It is well documented that these gastrointestinal side effects can lead to malabsorption of vitamin B12 in a dose- and time-dependent manner. Another medication used in the treatment of type 2 diabetes mellitus is sulfonylurea for example glibenclamide. Glibenclamide: Also known as glyburide is a medication used to treat diabetes mellitus type 2. Several soft tissue abnormalities have been reported to be associated with diabetes mellitus in the oral cavity. Burning mouth syndrome (BMS) affects 1.3 million Americans. Burning painful sensation in the mouth is often linked with dysgeusia and xerostomia. Classically, its symptoms improve in the morning, worsen during the day, and diminish at night.

**Aim and Objectives**

The aim of this study was to investigate the oral changes in salivary brain-derived neurotrophic factor (BDNF) in male patients with type 2 diabetes taking antidiabetic metformin as a monotherapy and comparing results with type 2 diabetic patients taking combination of metformin and glibenclamide. In addition to Clinical evaluation of the effect of the anti-diabetic medication in type 2 diabetes mellitus on burning mouth syndrome.

## Materials and Method

This case control study was conducted in the period from February 2019 to May 2019 after approval from Ministry of Health and College of Dentistry University of Baghdad by the scientific committee. The samples collection was done in the Endocrine center/ Merjan Teaching Hospital in Al- Hillah city/ Iraq during the period from December 2018 to the end of March 2019. Laboratory work was done by Al- Nasih clinical laboratory/ Baghdad- Iraq. After explaining the aims and the objectives of the study, written informed consent was obtained from all participants. Unstimulated saliva collected by draining method. The brain derived neurotrophic factor was estimated in saliva samples by using Human Brain derived neurotrophic factor (BDNF) ELISA kit.

Exclusion criteria: Excluded from this study female patient, Patient with systemic disease other than type 2 diabetes mellitus, and Patients take any medications other than metformin or the combination of metformin and glibenclamide.

Inclusion criteria: 80 male subjects Age >\= 40 years old included in this study divided in to three groups: 30 control healthy male without any systemic disease, 25 male patients with type 2 diabetes mellitus under metformin monotherapy 500 mg twice/day for at least 6 months ago, and 25 male patients under the combination of metformin 500 mg twice/day and glibenclamide 5 mg twice/day for at least 6 months ago.

Statistical analysis: Data were written in computerized database using Microsoft Excel (2016). Statistical analysis was performed with SPSS (Statistical Package for Social Sciences; Version 24) and Sigma Stat 4.0 software. Descriptive statistical analysis including: mean, standard deviation and standard error was extracted for each group, Analysis of Variance One Way-ANOVA on rank was done to identify differences among the study groups, and Post- hoc tests Bonferroni (multiple comparisons).

Results Regarding patients on metformin monotherapy treatment, the burning mouth syndrome seen in 6 (24 %) while in combination group seen in 5 (20%). Statistical analysis using ANOVA test showed that there is highly significant difference among study groups in relation to BMS, table (1).
Table (1): The Mean standard deviation of the burning mouth syndrome

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Mean BMS</th>
<th>±SD</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30</td>
<td>0.00</td>
<td>0.000</td>
<td>0</td>
</tr>
<tr>
<td>met</td>
<td>25</td>
<td>0.24</td>
<td>0.436</td>
<td>24%</td>
</tr>
<tr>
<td>met +g</td>
<td>25</td>
<td>0.20</td>
<td>0.408</td>
<td>20%</td>
</tr>
<tr>
<td>P value</td>
<td>0.01</td>
<td></td>
<td></td>
<td>HS</td>
</tr>
</tbody>
</table>

Post hoc revealed that there was increase in BMS in those patient on metformin treatment compared to control group with significant difference (p=0.029) and there is non-significant differences between patients on metformin monotherapy treatment and those on combination of metformin and glibenclamide treatment (p=1.000), table (2).

Table (2): Post hoc test of BMS among study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Group</th>
<th>Std. Error</th>
<th>P value</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
</tr>
<tr>
<td>control</td>
<td>met</td>
<td>.090</td>
<td>.029 S</td>
<td>-.46</td>
</tr>
<tr>
<td></td>
<td>met +g</td>
<td>.090</td>
<td>.089</td>
<td>-.42</td>
</tr>
<tr>
<td>met</td>
<td>control</td>
<td>.090</td>
<td>.029 S</td>
<td>.02</td>
</tr>
<tr>
<td></td>
<td>met +g</td>
<td>.094</td>
<td>1.000</td>
<td>-.19</td>
</tr>
<tr>
<td>met +g</td>
<td>control</td>
<td>.090</td>
<td>.089</td>
<td>-.02</td>
</tr>
<tr>
<td></td>
<td>met</td>
<td>.094</td>
<td>1.000</td>
<td>-.27</td>
</tr>
</tbody>
</table>

Also there is an increase in the mean and the standard deviation of BMI in both metformin and combination groups as compared to control group; Statistical analysis using ANOVA test showed there is significant difference of BMI among the study groups, table (3).

Table (3): Mean standard deviation and standard error of BMI for the study groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30</td>
<td>23.143</td>
<td>1.0575</td>
<td>.1931</td>
</tr>
<tr>
<td>met</td>
<td>25</td>
<td>23.632</td>
<td>1.4056</td>
<td>.2811</td>
</tr>
<tr>
<td>met +g</td>
<td>25</td>
<td>24.460</td>
<td>1.3354</td>
<td>.2671</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>23.708</td>
<td>1.3618</td>
<td>.1522</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>0.001</td>
<td></td>
<td>HS</td>
</tr>
</tbody>
</table>
Then by using post hoc Bonferroni test of BMI showed there is non-significant difference in those patient on metformin treatment compared to control group and those on combination of metformin and glibenclamide treatment show highly significant difference compared to control group in relation to BMI (p= 0.470, p=0.001) And there is non-significant difference between patients on metformin monotherapy treatment and those on combination of metformin and glibenclamide treatment in relation to BMI (p=0.069); table (4).

### Table (4): post hoc test Bonferroni of BMI among study group

<table>
<thead>
<tr>
<th>Group</th>
<th>Group</th>
<th>Std. Error</th>
<th>P value</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
</tr>
<tr>
<td>control</td>
<td>met</td>
<td>.3418</td>
<td>.470 NS</td>
<td>-1.325</td>
</tr>
<tr>
<td></td>
<td>met +g</td>
<td>.3418</td>
<td>.001 S</td>
<td>-2.153</td>
</tr>
<tr>
<td>met</td>
<td>control</td>
<td>.3418</td>
<td>.470 NS</td>
<td>-.348</td>
</tr>
<tr>
<td></td>
<td>met +g</td>
<td>.3570</td>
<td>.069 NS</td>
<td>-1.702</td>
</tr>
<tr>
<td>met +g</td>
<td>control</td>
<td>.3418</td>
<td>.001 S</td>
<td>.480</td>
</tr>
<tr>
<td></td>
<td>met</td>
<td>.3570</td>
<td>.069 NS</td>
<td>-.046</td>
</tr>
</tbody>
</table>

Results showed that there was increase in the mean and SD of BDNF in the two patients groups as compared to control group with overall mean= 0.576 SD ± 1.344. The mean of BDNF in control group 0.144 SD ± 0.069, in metformin group mean = 0.282 SD ± 0.125 and in combination group mean= 1.388 SD ± 2.217. ANOVA test showed that there was significant difference among the study groups in relation to salivary BDNF (p= 0.001), table (5).

### Table (5): Mean of salivary BDNF among study groups

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean ng/ml</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>30</td>
<td>0.14460</td>
<td>.069345</td>
<td>.012661</td>
</tr>
<tr>
<td>met</td>
<td>25</td>
<td>0.28264</td>
<td>.125050</td>
<td>.025010</td>
</tr>
<tr>
<td>met +g</td>
<td>25</td>
<td>1.38864</td>
<td>2.217081</td>
<td>.443416</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>0.57650</td>
<td>1.344138</td>
<td>.150279</td>
</tr>
</tbody>
</table>

P value 0.001 HS
Post hoc test showed there is non-significant difference in those patients on metformin treatment compared to control group in relation to BDNF (p=1.000) while there is significant salivary BDNF difference in combination group compared to control group (p=0.001) and there is significant difference in combination group compared to metformin group (p=0.007); table (6). Table (6): Post hoc Bonferroni test of salivary BDNF among the study group

<table>
<thead>
<tr>
<th>Group</th>
<th>group</th>
<th>Std. Error</th>
<th>P value</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>met</td>
<td>.335922</td>
<td>1.000</td>
<td>NS</td>
<td>-.96018</td>
</tr>
<tr>
<td>met +g</td>
<td>.335922</td>
<td>.001</td>
<td>HS</td>
<td>-2.06618</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>met</td>
<td>control</td>
<td>.335922</td>
<td>1.000</td>
<td>NS</td>
</tr>
<tr>
<td>met +g</td>
<td>.350859</td>
<td>.007</td>
<td>HS</td>
<td>-1.96469</td>
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<td>.335922</td>
<td>.001</td>
<td>HS</td>
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<tr>
<td></td>
<td>met</td>
<td>.350859</td>
<td>.007</td>
<td>S</td>
</tr>
</tbody>
</table>

Discussion

Diabetes mellitus is a wide spread complex disease with high morbidity and health care costs. Both diabetes mellitus and drugs used in the treatment of this disease can affect the mouth and salivary glands\textsuperscript{13}. The current study is the first study carried regarding salivary BDNF and burning mouth syndrome in type 2 diabetes mellitus under metformin as a monotherapy and combination of metformin and glibenclamide (comparative study). Burning mouth syndrome was seen in 24% of those patients with monotherapy and 20% in combination group and there is significant increase in the BMS in metformin monotherapy group as compared to control group and no significant difference between the monotherapy and the combination group. This study agreed with previous study of [Moore et al., 2007] that showed BMS or related discomforts occurred slightly more frequently than in the control group. Burning mouth syndrome (BMS) has been attributed secondarily to diabetes, poor glycemic control, and diabetic neuropathy. The correlation between diabetes mellitus and BMS is still controversial. It has been suggested that type II diabetes mellitus plays a role in BMS development and a link between the type of insulin used for the diabetes treatment and BMS has also been proposed. In contrast, other studies report a lack of association between these two conditions\textsuperscript{14}. A possible explanation for this controversy may be that these diabetic patients were erroneously classified as BMS. In fact, at the time of the above studies, a lack of strict criteria for BMS diagnosis could have affected the selection of the patients. For instance, burning oral complaints in diabetic subjects, who are more prone to oral infections, are probably caused by oral candidiasis. Although a large variety of drugs, medications, and miscellaneous treatments has been proposed in BMS, the treatment management of this syndrome is still not satisfactory, and there is no definitive cure. BMS patients have shown a good response to long-term therapy with systemic regimens of anti-depressants and anxiolytics\textsuperscript{15}. In the current study, there was a significant difference among studied groups regarding BMI. A significant increase in the BMI in the combination group as compared to control group was seen and there is non-significant difference between the two patients groups. This study showed that there is significant difference among the studied groups. Also there is significant
difference in combination group compared to metformin group. A significant increase in the mean of salivary BDNF in combination group as compared to metformin group was seen and there is no previous study regarding salivary BDNF in type 2 diabetic patient under therapy. In order to understand the impact of metformin on BDNF levels, [Ma et al. 2015] investigated the effect of metformin on Schwann cells under hypoxia condition and they found that the mRNA levels of BDNF were significantly decreased. However, this detrimental effect of hypoxia on gene expression in Schwann cells was partially reversed by metformin. The mRNA level of BDNF in metformin-treated Schwann cells was higher than those without metformin under hypoxia condition. This beneficial effect of metformin on gene expression under hypoxia condition was significantly inhibited by compound C, which is an inhibitor of AMP-activated protein kinase (AMPK) and an important cellular regulator of lipid and glucose metabolism16. Taken all together, these findings suggest that the correlation between BDNF and metformin may be the reason of metformin-induced insulin action by insulin receptor binding, metformin-induced high BDNF levels due to increasing AMPK, and enhanced tyrosine kinase receptor activity which may amplify BDNF signaling. BDNF inhibited during hyperglycemic clamp conditions in humans. This may explain the concomitant finding of low circulating levels of BDNF in individuals with type 2 diabetes17. Significant increase in the level of BDNF in combination group as compared to metformin group may explained by good glycemic control of combination treatment as compared to metformin mono-therapy treatment18.

**Conclusion**

Both monotherapy and combination therapy was affect salivary level of brain derived neurotrophic factor. Burning mouth syndrome was seen secondarily to diabetes and poor glycemic control and seen in both patients groups.

**Conflict of Interest:** We declare that we have no conflicts of interest.

Human and Animal Rights All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional research committee.

Informed Consent Informed consent was obtained from all individual patients included in this study.

**Source of Funding :** Self-funding

**References**

10. TING RZ, SZETO CC, CHAN MH, MA KK, CHOW KM. Risk factors of vitamin B(12) deficiency in patients receiving metformin. Arch


Efficacy of Chitosan Immune Response Against

Listeria Monocytogenes Infection in Mice

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Abstract

The present research aimed to study the effect of dietary chitosan supplementation against murine experimentally infection by Listeria monocytogenes.

forty mice were divided equally into 4 groups. The 1st and 2nd groups fed on diet supplement with chitosan (1mg/kg diet) and (1.5mg /kg diet) for (4) weeks respectively, While 3rd and 4th groups considered as control positive and negative groups. At (4) weeks the first three groups were inoculated intraperitoneally i/P with (0.2) ml (1×10^9) CFU/ml, while the 4th group (control negative) inoculated with (0.2) sterile normal saline.

At (7) days post infection, the result revealed diet one of mice in each control positive and treated group at (24hrs.) post infection with heavy bacterial isolation from brain, spleen and liver of infected positive group and mild to absent bacterial isolation in the 1st and 2nd group respectively.

Grossly presence of severe congestion in the internal organs with necrotic foci seen on the splenic surface of infected positive control while the characteristic feature in the treated infected group was hepatosplenomegaly.

Sever pathological changes were noticed in the infected positive control group characterized by suppurative inflammation with necrosis accompanied with lymphoid depletion and amyloid like substance deposition while the main lesion in treated infected groups showed granulomatous lesion, lymphoid hyperplasia and mononuclear cells infiltration with heavy bacterial isolation from brain, spleen and liver of infected positive group and mild to absent bacterial isolation in the first and second group respectively, We concluded that chitosan stimulated and improve the immune responses in mice against Listeria monocytogenes infection.

Key word: chitosan, Listeria monocytogenes, immunized, mice, pathology.

Introduction

Listeria monocytogenes is regular Gram-positive motile from, rod with rounded ends, its cells found as single units or short chains or may be arranged in V, L and Y forms or in palisades (1). Listeria monocytogenes does not produce spores and capsules are not formed (2). Spread in nature where, exists largely in decaying vegetation, soil, animal feces, feed and water as make it one of the major pollutants of food and play essential role in transmitted of infection between humans and animals (3) also infection by Listeria monocytogenes can be haematogenous spread directly from the mother to fetus (4, 5)

Chitosan is a modified natural carbohydrate polymer derived from chitin, it have many medical uses because their ability to reduce bleeding also help deliver drugs through the skin also in limiting of fat absorption(6), also has been bio adhesive property for that used as a safe excipient formulations of drug, it has been used in dentistry because adhere ability to hard and soft tissues also uses in orthopedics, ophthalmology and in surgical procedures, it adheres to epithelial tissues and mucus coat present on tissues surface also has a antifungal or antibacterial, antineoplastic and anticholestermic action(7).

Material and Method

Chitosan was obtained from university of Al-
Bahasa, collage of veterinary medicine. Commercial assorted pellets were grinded by food grinder and weighed (1) gm and (1.5) gm of Chitosan was added to each kilogram of grinded pellets mixed well and converted into paste which passed through meat grinder to mould the paste into the original pellets from, left exposed to dry in room temperature. The *Listeria monocytogenes* isolate was obtained from the unit of Zoonotic diseases in the College of Veterinary Medicine, the isolate confirmed by some biochemical tests and gram stain according to (9).

A total number (n=40) male white Swiss BALB/C mice which obtain from the (National Center of Researches and Drugs Monitor in Baghdad); then divided into fourth groups. The 1st group (n=5) mice were fed on diet supplement with chitosan (1mg/kg diet) and (1.5mg/kg diet) for (4) weeks respectively, While 3rd and 4th groups considered as control positive and negative groups. At (4) weeks the first three groups were inoculated intraperitoneally i/P with (0.2) ml (1×10^9) CFU/ml, while the 4th group (control negative) inoculated with (0.2) sterile normal saline, histopathological examination of internal organs (liver, spleen and brain) were taken from both control and infected groups about (1cm^3) was taken and fixed in 10% formalin saline for histopathological section which was done according to (10).

**Result and Discussion**

1) Gross pathological changes:

The main gross feature in control group was severe congestion in the visceral organs specially in the liver, spleen and kidney with presences necrotic foci at the edge of spleen, while treated groups show hepato-splenomegalgy was the characteristic gross lesion in the treated groups.

2) Bacterial isolate and clinical signs:

No clear clinical signs noticed on experimental animals specially the treated groups were appeared healthy and well feeding. The result showed heavy bacterial isolation mainly from brain, spleen and liver of control positive groups, while mild growth to absences in other treated groups. Also the isolate was confirmed again on blood agar then we made smear from isolate and stained with grams stain.

3) Histopathological examination:

The characteristic lesion in hepatic tissue of control positive show aggregation of PMNCs cells in liver parenchyma (suppurative foci) mainly in portal area accompanied with atrophy of some hepatic cords together with sinusoidal dilation and cellular infiltration in their lumen, The splenic tissue showed destructive changed with variable degree of lymphoid depletion in the white pulp, other section showed formation of multiple cystic cavities containing cellular debris together with focal amyloid like substances deposition, The brain tissue expresses sever neuronal degeneration and apoptosis accompanied with nuclear pyknosis and appearance of hypertrophic swelling astrocytes (gamistocyte), another section showed irregular cystic cavities with neuronal vaculation.

While the characteristic lesion in the liver of treated 1st group (fed on diet with 1gm/kg of chitosan) were development of early small granulomatous lesion seen in dilated sinusoids together with proliferation of kupffer cells (figure:1), the microscopic examination in the spleen revealed mild white pulp hyperplasia with proliferation of megakaryocyte (figure:2), together with slight vacuolar changes in some neurons also the results showed moderate gliosis (figure:3).

The pathological lesion in liver of treated 2nd group (fed on diet with 1.5gm/kg of chitosan) characterized by focal mononuclear cells (MNCs) aggregation mainly around central vein (figure:4) while presence of follicular hyperplasia in the white pulp was the main lesion observed in splenic tissue (figure:5), while the main brain lesion in the treated infected mice characterized by focal aggregation of MNCs in brain tissue, associated with no clear lesion in the neurons seen mainly in the brain section.

The present study shown sever pathological lesion in the internal organs (liver, spleen and kidney) of the control positive groups these results indicate that exposed to highly virulent microorganisms overcome the innate immune system and disseminates to internal organs induce tissue damage, these observation were in consistent with (11) who explained that virulent *Listeria monocytogenes* was one of intracellular bacteria disseminated via blood stream to internal organs and induce nonspecific inflammatory reaction by production listeriolysin O which destroyed the endothelial cells of blood vessels to induce necrosis and supplicative inflammation (12). In addition, survival and proliferation of microorganisms in the hepatic and splenic cells will
lead to the formation of infection foci that result the infiltration of a large number of WBCs and activate neutrophil phagocytic cells to work on other resist the invading germs (13). We also recorded depletion of white pulp of spleen of control positive group these observations may indicate that Listeria monocytogenes induced reduction in acquired immune response via depletion of lymphocytic cells (14). Neuronal necrosis and microcavities formation may due to excess of nitric oxide generation literal infection which is important for intracellular signaling of new transmission both inducible and constitute nitric oxide synthase (NoS) are expressed in brain cells include neural lesion, further more inflammatory cells include neutrophils, macrophages express both (NoS and iNoS) may play an important role in elimination Listeria monocytogenes (15). Also the present study explain that feeding infected mice showed mild to moderate pathological lesion in the spleen, liver and brain tissue post challenge with Listeria monocytogenes and these lesion characterized by appearance of granulomatous lesion mainly in liver tissue this evidence was agreement with (16) Where noted that the granulomatous reaction was considered the strongest body defense against virulent microorganism’s infection, furthermore there are numerous response indicate that chitosan improve the immune response (17). Our results showed lymphoid hyperplasia in splenic tissue mainly in mice feeding with (1.5gm/kg) chitosan this indicate that chitosan elicited both humeral and cell mediated immunity and activated immune cells to secret cytokines that play essential role in initiated mature granuloma in the liver and this evidence was in agreement with (18) Who demonstrated that feeding of chitosan increase OX62+ percentage and DCs which up regulate the major histocompatibility complex class-II Ags. without expression changing of co-stimulatory (CD80 or CD86) molecules and Ag presenting cells produced TNFα and IL-12 and activation T-lymphocytes, lymphoid tissue hyperplasia in animals fed diet supplement with chitosan may due to chitosan stimulated proliferation of lymphocytic cells.
Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References
Fatal Drowning in Delta State, Nigeria: A Retrospective Study of Cases in this Region

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Abstract

Introduction: Drowning death is a preventable, under-reported public health problem resulting from respiratory insufficiency secondary to immersion or submersion in liquid.

Aim: To study the sex, age, and place of death of victims of fatal drowning in Warri, Delta state, Nigeria.

Material and Method: This is a descriptive, retrospective study of cases of fatal drowning reported to the coroner and subjected to postmortem examination by the authors in Delta State from 1st January 2003 to 31th December 2016. Basic information such as the age, sex and place of death were extracted and analyzed using Microsoft Office Excel, version 2007.

Results: Thirty-seven victims comprising of 34 males and 3 females were examined during this study, giving a mean incidence of about 2.5 cases per annum. Their ages ranged from 1.5 to 59 years with a mean of 28.53 years and a dual peak in the 3rd and 4th decades. All the deaths were of accidental causes, with most the of them (70%) occurring within natural water bodies.

Conclusion: The study showed that drowning death is relatively common and usually of accidental etiology. Young males in their thirties and forties are the most vulnerable victims, with the natural water bodies being the most common site of drowning. Being a preventable cause of death, adopting and enforcing preventive safety measures by the individuals, community and government will invariably reverse this trend.

Key word: Drowning, Medicolegal, Preventable death

Introduction

According to statistics from WHO, unintentional injuries account for 3.9 million deaths annually with about 90% of these cases occurring in low and middle income countries (LMIC). These are attributed mostly to road traffic accident (RTA), fall, drowning, poisoning and burn.1

Drowning is currently defined as “the process of experiencing respiratory impairment from partial or complete submersion/immersion in liquid”. Its outcome may be fatal (death) or non-fatal, the latter of which may be with or without morbidity.2

Though preventable, fatal drowning has become a serious public health issue globally ranking among the three leading causes of injury related death in most countries.3 According to WHO report, drowning accounted for about 372,000 deaths in 2012, with 91% of these deaths occurring in LMIC.4 Children have been shown to be particularly susceptible with about 450 dying daily from drowning, and a significant number suffering from varying grades of morbidities.5 Sadly, the African continent accounts for the highest drowning mortality with a rate 13.1 per 100,000 population.6

Delta State of Nigeria is unique because of numerous unprotected natural water bodies with 35% of its 16,842 square kilometers total land area being riverine. It also has a high density of streams, ponds, lakes, creeks as well as the large body of ocean waters.7
Despite the burden of drowning death, there is paucity of research on this subject matter in this region. This study is aimed at analyzing the age, sex and death-place of victims of fatal drowning, examined by the authors during the study period. Being the earliest of such study in this part of the world, we hope the findings will increase public and government awareness of magnitude of the problem, guide government policy formulation, contribute to literature and lastly suggest direction for subsequent research.

**Materials and Method**

This is a 14-year descriptive retrospective study of drowning deaths recorded by the authors in Warri, Delta State from January 2003 to December 2016.

All medicolegal autopsies performed in this region by the authors were reviewed and confirmed cases of drowning identified for this study. The information used for this study includes the age, sex, circumstance and site of death of the victims. This information was subsequently analyzed using excel spread sheet and presented in tables.

Exclusion criteria: All cases of post-mortem drowning as well as cases of doubtful history were excluded from the study.

**Result**

In this retrospective study, 1121 medicolegal autopsies were analyzed, out of which 37 (3.3%) of the cases were as a result of drowning.

The age distribution of victims is shown in table 1, ranging from 1.5 years to 59 years, with a mean age of 28.53 years and two unimodal peaks occurring in the 3rd and 4th decade.

The sex distribution of the victims is shown in table II, with males and females accounting for 34 (91.9%) and 3 (8.1%) of the cases respectively.

Table III showed the places where the drowning occurred. Natural waters (Oceans, creeks, rivers, streams and lakes) were the site of occurrence in 26 cases (70.3%). Interestingly, two of these deaths took place during immersion baptism. Three cases (8.1%) occurred in water well. Coincidentally, two cases each (5.4%) were recorded in four different setting during the study namely: fish pond, pipeline excavation pits, gas tanks and swimming pools.

**Table I Age distribution of victims of death by drowning**

<table>
<thead>
<tr>
<th>Age of victims (years)</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>2</td>
<td>5.4</td>
</tr>
<tr>
<td>11-20</td>
<td>6</td>
<td>16.2</td>
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<tr>
<td>21-30</td>
<td>12</td>
<td>32.4</td>
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<tr>
<td>21-40</td>
<td>12</td>
<td>32.4</td>
</tr>
<tr>
<td>41-50</td>
<td>3</td>
<td>8.1</td>
</tr>
<tr>
<td>51-60</td>
<td>2</td>
<td>5.4</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table II Gender distribution of the Victims**

<table>
<thead>
<tr>
<th>Gender of victims</th>
<th>Frequency</th>
<th>Percentage (%)</th>
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</thead>
<tbody>
<tr>
<td>Males</td>
<td>34</td>
<td>91.9</td>
</tr>
<tr>
<td>Females</td>
<td>3</td>
<td>8.1</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>100</td>
</tr>
</tbody>
</table>
Table III: Place of Occurrence of fatal drowning

<table>
<thead>
<tr>
<th>Place of Occurrence</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish pond</td>
<td>2</td>
<td>5.4</td>
</tr>
<tr>
<td>Gas tank</td>
<td>2</td>
<td>5.4</td>
</tr>
<tr>
<td>Excavated Pit</td>
<td>2</td>
<td>5.4</td>
</tr>
<tr>
<td>Natural bodies of water</td>
<td>26</td>
<td>70.3</td>
</tr>
<tr>
<td>(Rivers/Lakes/Creeks)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swimming pool</td>
<td>3</td>
<td>8.1</td>
</tr>
<tr>
<td>Wells</td>
<td>2</td>
<td>5.4</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>100</td>
</tr>
</tbody>
</table>

Discussion

Thirty seven (3.3%) of the medicolegal deaths in the study population were drowning death. This figure is intermediate between 2.2% reported by Nwafor and Akhiwu in Benin City, Nigeria and 4.3% reported by Ngbea et al in Makurdi, Nigeria.

We are of the opinion that this is a gross underestimation of its burden because of endemic inefficiency at data collection often seen in developing countries, and the poor attitude towards reporting accidental deaths to the police. The trend of practice where relatives of the deceased are made to pay for the autopsy has also not encouraged these relatives to report such cases to the police.

This study showed that males were found to be 11.3 times at higher risk, than the females. The high male to female ratio observed in this study concurs with reports from 62 articles in English literature reviewed on this subject matter in which a mean male-female ration (MFR) of 3:1 was observed. This male predominance may be attributed to their greater involvement in boat-driving and other boat-centric jobs, including fishing and trading. Inherently, the males are more aggressive, competitive, and take on to riskier activities and behaviors some of which include alcohol, drug abuses and swimming alone, all of which may further increase the risk of drowning. Activities in the water bodies such as crude oil theft and sea piracy which are common activities in this region may also doubt contribute to this ugly trend.

A double unimodal peak was observed in the 3rd and 4th decade, with each peak representing 32.5% of the cases. This is most likely as a result of the active lifestyle of this age group. Our observation compares favorably with those from Benin City, Nigeria, where the highest number of victims were in their 3rd decade, but contrasts with report from an earlier study in the Niger Delta region which recorded the highest number of cases in the 6th decade of life. Drowning death risk is highest among children globally. In the index study, 18.9% of the victims were children, majority of which were in their 2nd decade of life. This is lower than earlier report among children in Benin City and Niger Delta region. Lapses in supervision especially from parents has always been the major explanation to drowning among children. The lower incidence of childhood drowning death in Nigerian series, relative to the global trend, may be attributed to case underreporting as natives in the study population attach less significance to death of children than that of adults.

Environmental factors, culture, behavior of the people as well as the geography of the environment play remarkable roles in the setting of drowning death. Delta state has a rich network of natural body waters, with a lot of transportation, recreational, fishing and trading activities. This may explain the occurrence of most cases of drowning death in these natural water bodies. Our report is similar to findings in Bangladesh with 95% of cases reported in a similar setting. Likewise, Sheikhaazadi in Iran, and Pal et al in India reported 83% and 81.8% cases respectively in fresh water setting.
Our study also concurs with an earlier report by Seleye-Fubara in Niger Delta region.\textsuperscript{12} On the contrary, Nwafor et al reported lower frequency of drowning death in natural water bodies in Benin, a city that has fewer rivers within its geographic boundaries.\textsuperscript{11}

The two cases of drowning during baptism recorded in this study is a cautionary call to the church as it underscores the imminent danger of such activities. There is need to re-orient the church to avoid such practices where possible and if unavoidable, to adopt preventive measures including use of protective devices and employing divers to avert grave consequences. Arresting and prosecuting church leaders will act as a deterrent to others.

Public swimming pools were the second most common site (8.1\%) of drowning death in this study. This is a reflection of proliferation of swimming pools in modern hotels and bars, probably an influence of westernization of our culture. We think this is mainly as a result of misadventure and recklessness of the victims, who are predominantly young. It is a general observation that these setting encourage alcohol and drug use, the role of these substances in the risk of drowning may not be overemphasized. Earlier reports have shown that swimming under the influence of alcohol is associated with increased risk of drowning death.\textsuperscript{13} In USA, the highest rate of drowning occurred in swimming pools, which disagrees with our report.\textsuperscript{15} In Japan, the highest drowning death occurred in bathtubs, mostly affecting the elderly persons.\textsuperscript{16} Enforcement of fencing of Public swimming pools and use of personal floating devices routinely are worthwhile preventive measures.

Wells, fish ponds, pits and gas tanks accounted for the remaining settings for drowning death in this study. These settings are all products of man’s activities and interference with the natural environment. Therefore, using barrier fencing, caution signs, early filling up of ditches and pits, especially at sites of road construction will no doubt prevent such accidents.

With respect to forensic etiology, accidental drowning accounted for all the cases. Sheikhazadi in Iran,\textsuperscript{13} Nwafor in Benin, Nigeria,\textsuperscript{8} and Seleye-Fubara in Niger Delta region, Nigeria\textsuperscript{12} observed that most cases were of accidental causes. The rarity of homicidal drowning may not be unconnected with the difficulty in drowning a healthy conscious adult. As in our study, suicidal drowning was not reported in other Nigerian studies.\textsuperscript{8,12,13} Though there has not been any study on suicide in this region, earlier studies among Nigerian Yorubas (a major ethnic group in Southern Nigeria) showed a strong negative attitude towards Suicide.\textsuperscript{17}

In conclusion, the study showed that drowning death is relatively uncommon and all of accidental etiology in this study. Young males in their thirties and forties are the most vulnerable victims, with the natural water bodies being the most common site of drowning. Being a preventable cause of death, adopting and enforcing preventive safety measures by the individuals, community and government will invariably reverse this trend.

**Limitation of the study:**

The relatively small sample size, under-reporting of the cases, the effect of co-morbid conditions, drugs and alcohol may interfere with the outcome of this report.

**Conflict of Interest:** Nil

**Source of Funding:** Self

**Ethical Clearance:** Ethical approval was obtained from the ethical clearance committee of Delta State Hospital management Board/Central Hospital, Warri (reference CHW/ECC VOL1/124).

**References**


Measurement of Effective Dose Detox for Workers Exposed to Benzene Toxins in Sidoarjo Paint Industry

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Abstract

One of the aromatic hydrocarbon compounds, namely benzene. Benzene is a colorless liquid with a sweet odor (sweet odor), volatile in the air, soluble in water and flammable. Today the use of benzene used by the paint manufacturing industry. However, the concentration of benzene that exceed standards required threshold value will negatively impact the health of workers in Sidoarjo Paint Industry. Benzene can threaten the safety and human health if inhaled because it can damage the blood forming system profile in humans. The purpose of this research is to reduce the exposure of benzene by determining the effective dose of toxin detox benzene in the paint industry, Sidoarjo, East Java. This study was an observational study with cross-sectional approach. Result the average concentration of benzene in the workplace respondents was 3.28 mg/m³. The average effective dose should be consumed by the respondent for the avocado intake is 0.011 mg/day, chicken intake 0.026 mg/day, intake of grapefruit 0.033 mg/day, and carrot intake was 0.063 mg/day.

Keywords: Benzene, an effective dose of detox, paint factory worker

Introduction

One of the aromatic hydrocarbon compounds, namely benzene which has many uses for human life, especially in the industrial sector14. Epidemiological studies prove benzene as a carcinogen substance, have toxic effects on the blood and bone marrow. The International Agency for Research on Cancer (IARC) classifies benzene into the group-1A carcinogen material, a material that proved to be carcinogenic to humans. Contained toxic levels of benzene contained in the human circulatory system can cause leukemia2. NIOSH (National Institute of Occupational Safety and Health) said that workers exposed to organic solvents, it is estimated to reach 9.8 million people, mainly workers who work on the production of paints, adhesives, glues, coatings, grease solvent / cleaning materials, production dyes, polymers, plastics, textiles, printing inks, agricultural products, and pharmaceuticals12. NIOSH estimates that more than 2 million workers in the United States the possibility of exposure to benzene.1 In Indonesia, definitive data regarding the effects of benzene on workers association has not been found14.

Benzene includes components of gasoline and toxins commonly regarded as the work16. Benzene is a toxic chemical that can lead to acute myeloid leukemia10,12. Benzene when oxidized in Phase 1 will produce benzene quinone, which is a source of increased toxicity. To complete benzene required in Phase 2 detoxification enzymes CYP2E111. metabolic enzymes play an important role in the activation or detoxification benzene15. Benzene is initially oxidized to benzene oxide by the liver cytochrome P450 2E1 (CYP2E1) in the liver14.

BO detoxification in vivo occurs by reaction with glutathione, some metabolites of benzene aromatic rings opened or catalyzed by enzymes II metabolites such as glutathione-S-transferase (GSTs) or to form the derivative is less toxic or non-toxic and excreted through the urine17. To reduce or even eliminate toxins in the chemical, diperlukan4 biotransformation process. High levels of antioxidants needed to neutralize these substances, compensate for the damage caused by free radicals and protects the liver and cells of the CYP2E111. Foods rich in beef liver, brain, and salmon; Sulfation

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rich foods are eggs, sulfation of chicken, beef and tuna, and foods rich in glutathione are avocado, asparagus, carrots, tomatoes, oranges, and broccoli.

Research using food to detoxify benzene is still very limited. Therefore, this study aimed to determine the CYP2E1 enzyme-rich food intake, sulfation, and glutathione required to detoxify benzene in every worker in Sidoarjo Paint Industry.

Benzene is one of the aromatic hydrocarbon compounds are widely used in the rubber industry, oil refining, chemical plants, shoe factories, and oil-related industries. Benzene is also found in public facilities such as cigarette smoke, gas stations, fuel burning cars, and so on. According to the Agency for Toxic Substances and Disease Registry (ATSDR), hazardous and toxic chemicals contained in oil content, namely benzene, toluene, xylene, ethylene, TPH (Total Petroleum Hydrocarbon) and Polycyclic Aromatic Hydrocarbon (PAHs). Of the six chemicals benzene exposure are very serious impact on health.

American Conference of Government Industrial Hygienists (ACGIH) in 2006 set the Threshold Limit Value (NAV) of the chemical benzene in the workplace that is the maximum value allowed is 0.5 ppm and certainly belonged carcinogenic to humans (A1 = Confirmed Human carcinogen) 14. National Institute for Occupational Health and Safety (NIOSH) set the recommended exposure limit or REL (recommended exposure Limit) for 8 hours of work that is equal to 0.1 ppm. In Indonesia alone, according Permenakertrans 13 / MEN / X / 2011, 2011, NAB allowable benzene is 0.5 ppm.6 While based on the Minimum Risk Level (MRL) set for the ATSDR 2007 was 0.009 ppm benzene every day to give effect acute and 0.003 ppm every day cause chronic effects 2.

Areas of the paint industry as a working environment that has a high exposure to benzene, supposedly necessary efforts to reduce the negative effects of exposure to benzene so as not to cause health problems for workers. Based on previous studies of benzene in the workplace, has not done research on effective dose detok toxic benzene in the workplace that have exposure to benzene. Therefore, the authors are interested in discussing the size of an effective dose of toxic benzene in the area detok paint industry.

Material and Method

The subjects are workers in the paint industry Sidoarjo. Criteria for inclusion in this study were male workers who had worked in the paint industry and are willing to be used as respondents. The sample was 24 people.

The variables that need to be calculated first is the effective dose intake needed by each individual to calculate the weight, length of employment (years), worked on average each day (hour), and the working time in a week (days) of the respondents, as well as the measurement of the concentration, benzene at five points in the industry. Weight measurement using the scale body weight. Working length measurement, the average work every day, and time workweek obtained through in-depth interviews with respondents. Then, the measurement of the concentration of benzene in the workplace by using the NIOSH 1501 method of measurement with activated charcoal carbon pipe using the technique of gas chromatography (NIOSH 1501, 2003). The study was approved by the Ethics Committee of the Faculty of Public Health.

Then, look for the intake (formula) with the following formula:

\[
\text{intake non carcinogen} = \frac{C \times R \times tE \times fE \times Dt}{Wb \times 30 \times 365}
\]
C = concentration of benzene (mg / ml)
R = benzene reaction rate (m3 / h)
te = working time / day (hours)
Fe = working time / week (day)
Dt = working time (years)
Wb = weight (kg)

Calculate the effective dose per day with the calculation results noncarcinogen intake (intake) above, using the formula below:

\[
effective
dose
dead
= \frac{intake}{days}
= \frac{intake}{\text{toxin day}} \times \frac{Mr
enzym
detox}{Mr\ toxin}
\]

Findings

Benzene concentration (n = 24)

In Figure 1, shows that the benzene concentration maximum of 6.7508 mg / m3 and the lowest of 1.1975 mg / m3. So that the average benzene in Sidoarjo paint industry was 3.28 mg / m3.

Comparison of Predicted Benzene intake Detox
Table 1. Comparison of Predicted Benzene Detox intake (Intake of Beef Liver, Brain Cow, Fish Salmon) (n = 24)

<table>
<thead>
<tr>
<th>C (Mg / m³)</th>
<th>DE Avocado</th>
<th>DE Chicken</th>
<th>DE Orange</th>
<th>DE Carrot</th>
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<tr>
<td>6.7508</td>
<td>0.028338827</td>
<td>0.067493999</td>
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<td>0.081832912</td>
<td>0.101133127</td>
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<tr>
<td>6.7508</td>
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<td>0.083697317</td>
<td>0.103437251</td>
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<tr>
<td>6.7508</td>
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<td>0.033305622</td>
<td>0.063042784</td>
</tr>
</tbody>
</table>
In Table 1 and Figure 2, shows that the highest benzene concentration of 6.7508 mg / m3 and the lowest of 1.1975 mg / m3. So that the average benzene in Sidoarjo paint industry was 3.28 mg / m3. The average effective dose should be consumed by the respondents to the intake of avocado is0,011 mg / hr, chicken intake of 0,026 mg / day, The intake of citrus 0,033 mg / hr, and carrot intake was 0.063 mg / hr.

**Discussion**

Detoxification is the process that is toxic to less toxic compounds. The detoxification process to be non-toxic eliminated through urine and bile. Food-based nutrition continues to be investigated for its role in modulating the metabolic pathways involved in the detoxification process. Some of the publications that have used the cell, animal and clinical studies indicate that the component-based foods and nutrients can modulate the process of conversion and excretion of toxins from the body.

YP2E1, Sulfation, and glutathione are enzymes that work on phase 2 of the biotransformation 9. CYP2E1 are involved in detoxification reactions-activation of a variety of endogenous and exogenous compounds. Rat hearts showed that the effect of the amount of protein in CYP2E1 activity increased CYP2E1 enzyme activity increased 2.6. Sulphation is one of a number of liver detoxification pathway, particularly the phase II detoxification. The sulphation detoxification of toxins, it is an antioxidant and detoxification compound produced a powerful agent in the cytoplasm of every cell of the human body 13.

Related glutathione antioxidant enzyme involved in the metabolism and detoxification of cytotoxic and carcinogenic compounds as well as reactive oxygen species.

The generation of reactive oxygen species occur in relatively prolonged hypoperfusion conditions such as aging. The etiology of presbycusis is much less certain; However, the genetic cause is most likely complex. The effects of aging shows a variety of inter-wide.

The results showed that each individual has a different cost. This is because each individual has a different effective dose of food. The effective dose can also depending on the amount of inhaled benzene concentration, weight, and length of employment. The higher the concentration of benzene in the body, the greater the mass of food needed detox. This is in
accordance with the formula that has been made in previous studies suggesting that he has a synergistic relationship with the concentration of weight, length of employment, and the concentration of benzene can affect the intake of non-carcinogens in each individual that can influence the effective dose of food. This is consistent with previous research that says that the genetic variance, sex, and weight may play a role in the biotransformation enzymes.

By knowing the foods that can be used to detoxify benzene exposure of the body, in the paint industry Sidoarjo who have a high risk of exposure to benzene can prevent this is through food. In addition, knowing the estimated costs to be incurred in order to prevent exposure to benzene through this food, workers can choose foods that can detoxify benzene in the body with the food that is in line with workers’ earnings in the paint industry Sidoarjo.

Sulphation is one of a number of liver detoxification pathways, particularly the phase II detoxification. Sulphation system is important in detoxification of several drugs, food additives and especially the gut bacteria and toxins from environmental contaminants. Glutathione is an antioxidant and detoxification compound produced a powerful agent in the cytoplasm of every cell of the human body. In broad terms, these studies have found glutathione to protect against oxidative stress, detoxification of chemicals and toxins, improve immune function, and support healthy aging. One of the toxins that can be detoxified is benzene.

The results showed that each individual has different needs detoxification intake. This is because each individual has a different effective dose of food. The effective dose can also depend on the amount of inhaled benzene concentration, weight, and length of employment. The higher the concentration of benzene in the body, the greater the mass of food needed detox. This is in accordance with the formula that has been made in previous studies suggesting that he has a synergistic relationship with the concentration of a substance. Weight, length of employment, and the concentration of benzene can affect the intake of non-carcinogens in each individual that can influence the effective dose of food. This is consistent with previous research that says that the genetic variation, sex.

By knowing which foods can be used to detoxify the body’s exposure to benzene from, shoe workers who have a high risk of exposure to benzene can prevent this is through food. In addition, knowing the estimated costs to be incurred in order to prevent exposure to benzene through this food, workers can choose foods that can detoxify benzene in the body with the food that is in line with workers’ earnings in the paint industry Sidoarjo.

Conclusion

To complete the detoxification benzene required in Phase II enzyme CYP2E1 enzyme necessary, sulfation, and glutathione. CYP2E1-rich foods are beef liver, brain, and salmon; Sulphation rich foods are eggs, chicken sulfation, beef and tuna, and foods rich in glutathione are avocado, asparagus, carrots, tomatoes, oranges, and broccoli. Weight, length of employment, and the concentration of benzene can affect the intake of non-carcinogens in each individual that can influence the effective dose of food. By knowing the estimated costs to be incurred in order to prevent exposure to benzene through this food, workers can choose foods that can detoxify benzene in the body with the food that is in line with workers’ earnings in the Paint Industry Sidoarjo.

Consequences of benzene exposure in workers obtained from the use of benzene which is very much needed and important role in many industrial processes. This can negatively impact the health of workers if efforts to control or reduce the exposure is not done well. Karsinogenen effect is one of the harmful effects resulting from exposure to benzene in the workplace, especially in the paint industry.

Therefore, the average benzene in Sidoarjo paint industry was 3.28 mg/m3, the average effective dose should be consumed by the respondents to the intake of avocado is 0.011 mg/hr, chicken intake of 0.026 mg/day, The intake of citrus 0.033 mg/hr, and carrot intake was 0.063 mg/hr. So that to reduce the impact of exposure to benzene into the body of the workers in the paint industry needed feeding benzene detoxification. Feeding is different in each individual adjusted to the concentration of benzene, weight, and duration of action.

Conflict of Interest: The authors declare no conflict of interest.

Source of Funding: The source of this research costs from the self.

Ethical Clearance: The study was approved by the Ethics Committee of the Faculty of Public Health,
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Salivary Zinc level and Taste Detection Thresholds in Hypertensive Patients on Amlodipine and on Losartan (A Comparative Study)

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Abstract

High blood pressure (hypertension) is one of greatest risk factors for cardiovascular disease, which is a remarkable cause of morbidity and mortality worldwide. The outcome of hypertension (HTN) and cardiovascular disease (CVD) is influenced by a wide variation of risk factors like use of tobacco; excessive alcohol consumption; unhealthy diet, few physical activity, overweight and obesity; high blood glucose, and abnormal blood lipids. The objectives of this study were to evaluate taste detection thresholds (of four basic tastes) of hypertensive patients on Amlodipine 5 mg and on Losartan 50 mg. And to estimate Zinc level in saliva of those patients and compare it with control subjects. A total of 90 subjects were incorporated in this study they were divided into three groups: 1-Thirty patients on Amlodipine (5mg) 2-Thirty patients on Losartan (50mg) and 3- Thirty healthy control subjects. Unstimulated whole saliva was collected from all subjects including in this study. Volume of 5 ml of each taste gradient solution was offered to the participants. The samples were subjected to biomechanical analysis to estimate zinc level by using the atomic absorption spectrophotometer. The result showed that the taste detection threshold of sucrose and salt were significantly higher in patients on Amlodipine and on Losartan treatment than in control subjects. While the taste detection threshold of sour and bitter showed no significant differences between the study groups. Also there is a alteration in salivary Zinc, Zinc in patients on Amlodipine and on Losartan was significantly lower compared to control group.

Keywords: Taste Detection Thresholds, Hypertensive, Amlodipine, Losartan, Zinc

Introduction

Hypertension is often called a silent killer since frequently there are no clear symptoms. Initial signs of hypertension may be non-specific (headaches, excessive irritability, insomnia, decreased tolerance to exercise, palpitations and flushing of the head, neck and chest) and thus confusing¹. Hypertension has lately been reaffirmed as the major single risk factor contributing to world death rates². Besides, control of hypertension is one of the most cost-effective process to decrease early cardiovascular morbidity and mortality³. The national institute for health and care excellence(NICE) recommended that management of high blood pressure with any of many classes of common medication is cost saving compared with providing of no medication. The aims of management of hypertension is to control arterial pressure, prevent end-organ damage (cardiovascular, cerebrovascular, and renal), and to decrease the chance of premature death⁴.

Classification of drugs may be done by mechanism or site of action (therapeutic british hypertension society, 2014). In each class, there are numerous drugs with variation in structure and pharmacology resulting in alteration in therapeutic and side-effects⁵.

Amlodipine is an oral dihydropyridine calcium channel blocker. Compared to nifedipine and other medications in the dihydropyridine class, amlodipine
has the longest half-life at 30 to 50 hours. The advantage of such a long half-life is the ability to have once-daily dosing. Amlodipine (dihydropyridines) has been described to be an efficient antihypertensive medication related with regression of left ventricular hypertrophy and vascular hypertrophy, the antiatherogenic and the remodeling effects.\(^6\)

Losartan is an angiotensin II receptor antagonist (AIIRA) with antihypertensive action due mainly to selective blockade of (AT1) receptors and as the consequence reduced the effect of angiotensin II in elevation of blood pressure. Losartan use in the management of hypertension and heart failure, especially in patients who develop cough with the use of Angiotensin converting enzyme (ACE) inhibitors, additionally it is used in patients with left ventricular hypertrophy to reduce the risk of stroke, and also used in the management of diabetic nephropathy, and has been tried in management of myocardial infarction.\(^7\) Peak plasma concentrations of losartan are achieved within one hour of oral administration the half-life of Losartan 1.5–2.5 hours.\(^8\)

Taste is an important protective sense, progressed to manage the intake of food and help in the avoidance of poison. Genetic considered as the principal determinants of taste threshold, and taste thresholds do not differ significantly from day to day. This has lead to the idea of “non-tasters” and “supertasters”\(^9\), in which the taste threshold is linked to the haplotype (a combination of alleles for different genes and tend to be inherited together) of specific receptors.\(^10\) Taste disturbances may arise secondary to autoimmune disease, inflammation, imbalance of hormone, nerve-related damage, psychological problems like in anorexia, medication therapy or malignancy; they may also occur as a consequence of natural aging.\(^11\) Zinc is an essential trace element and is found in tissues throughout the body, reaching iron in its relative abundance.\(^12\) The body of human carry about 2g of zinc, approximately 60% of which is found in muscle tissue, 30% in bone and 5% in skin.\(^13\)

**Aims and Objectives**

1- To Evaluate taste detection thresholds (of four basic tastes) of hypertensive patients on Amlodipine (5 mg) and on Losartan (50 mg).

2- To estimate Zinc level in saliva of those patients and compare it with control subjects.

**Materials and Method**

This case control study was conducted in the period from February 2019 to May 2019. After approval from Ministry of Health and College of Dentistry University of Baghdad by the scientific committee. A total of 90 subjects were incorporated in this study, they were divided into three groups: 1- Thirty patients on Amlodipine 5mg. 2- Thirty patients on Losartan 50 mg. 3- thirty healthy control subjects.

**Inclusion criteria:** All patients presented with hypertension and they were under antihypertensive monotherapy for at least 8 months. The antihypertensive medications that used by the patients are: Amlodipine and Losartan.

**Exclusion criteria:** hypertensive patient taking combination of antihypertensive medications, Diabetes mellitus, Renal failure, smoking, patients with history of radiotherapy in the head and neck region, and history of chemotherapeutic treatment in the last 3 months.

Each taste gradient consisted of 15 solution, from 1.5 to 15.5 mmol (in 1 mmol increments) for sucrose, from 1-78 mmol (in 5.5 mmol increments) for sodium chloride, from 48-720 µmol (in 48 µmol increments) for citric acid, and from 89-117 mmol (in 2mmol increments) for urea. \(^14\) Taste solution were Prepared by calculation the amount of taste substance in grams dissolved in deionized water for recommended concentration according to weight (g)= molecular weight(g/mole) x concentration(M) x volume(ml)/1000. \(^15\) The concentrations of solutions used are shown in table (1). The sip and spit method was used, the taste solution were swirled around in the mouth briefly and expectorated into an empty cup. \(^16\)
Table (1): The concentration of taste solutions used (Amerine and Pangborn, 1965)\textsuperscript{14}

<table>
<thead>
<tr>
<th>Sucrose mmol/L</th>
<th>Sodium chloride mmol/L</th>
<th>Citric acid μmol/L</th>
<th>Urea mmol/L</th>
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**Statistical Analysis**

The statistical package for the social sciences (SPSS) was used for data input and analysis. The statistical significance of difference in mean between more than two groups was assessed using the ANOVA model. When statistically significant difference was shown by the use of ANOVA, further exploration of the statistical significance of difference in mean between each two groups was assessed by post-hoc multiple comparison Least Significant Difference (LSD) test.

**Results**

This study showed that the mean and standard deviation of the taste detection threshold of sucrose (sweetness) of patients on Amlodipine was 14.16±2.48 mmol/l, and those on Losartan treatment was 12.76±3.20 mmol/l. For the control subjects was 8.16±2.48 mmol/l. It has been found that the taste detection threshold of sweet showed significant difference (p<0.01) using ANOVA test and was highest among patients on Amlodipine (Table 2). Continuing analysis with (LSD) test showed that the taste detection thresholds of sweetness was significantly higher in patients on Amlodipine and on Losartan treatment than in control
subjects (p<0.00 1) but no significant difference has been found between patients on Losartan and patients on Amlodipine (Table 3).

The mean and standard deviation of the detection threshold of salty taste of patients on Amlodipine patients was 52.90± 12.03 mmol/l , and on Losartan treatment was 55.83± 14.94 mmol/l , while in control subjects was 32.11±21.66 mmol/l. It has been shown that the detection threshold of salt was significantly higher in patients on Amlodipine and Losartan treatment than that in the control subjects (Table 2). Continuing analysis with (LSD) test, showed that the taste detection thresholds of salt in patients on Amlodipine was significantly higher than that of control subjects. And the taste detection thresholds of salt in patients on Losartan was significantly higher than that of control subjects , but no significant differences was found between patients on Amlodipine and patients on Losartan (Table 3).

For the sour taste, the results showed that the mean and standard deviation of the detection threshold for citric acid in Amlodipine patients was 473.43±183.91 μmol/l and of patients on Losartan treatment was 453.23 ±175.59 μmol/l, while in control subjects the detection threshold for citric acid (sourness) was 515.46±192.34 μmol/l (Table 2). Statistical analysis showed no significant differences between the study groups.

For the bitter taste, it has been shown that the mean and standard deviation of urea (bitterness) in patients on Amlodipine patients was 91.75± 21.09 mmol/l, and for Losartan treatment was 85.00 ± 26.32 mmol/l, while for control subjects was 92.20±17.51 mmol/l (Table 2). No significant differences was found between the study groups.

Table (2):- The mean and standard deviations of the taste detection threshold of the four tastes in study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Sweet</th>
<th>Salt</th>
<th>Sour</th>
<th>Bitter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Amlodipine</td>
<td>14.16</td>
<td>2.48</td>
<td>52.90</td>
<td>12.03</td>
</tr>
<tr>
<td>Losartan</td>
<td>12.76</td>
<td>3.20</td>
<td>55.83</td>
<td>14.94</td>
</tr>
<tr>
<td>Control</td>
<td>8.16</td>
<td>2.48</td>
<td>32.11</td>
<td>21.66</td>
</tr>
<tr>
<td>P. value</td>
<td>0.00**</td>
<td>HS</td>
<td>0.00**</td>
<td>HS</td>
</tr>
</tbody>
</table>

All units in mmol/L except for sourness in μmol/l

** HS:Highly significant p<0.001    NS: - None significant p> 0.05

Table (3): - Multiple comparisons using LSD among study groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Subgroups</th>
<th>Std. Error</th>
<th>P. Vlue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweet</td>
<td>Amlodipine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Losartan</td>
<td>.70857</td>
<td>0.051</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>.70857</td>
<td>.000 HS</td>
</tr>
<tr>
<td></td>
<td>Losartan</td>
<td>.70857</td>
<td>.000 HS</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>.70857</td>
<td>.000 HS</td>
</tr>
<tr>
<td>Salt</td>
<td>Amlodipine</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Losartan</td>
<td>4.3147</td>
<td>0.498</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>4.3147</td>
<td>.000 HS</td>
</tr>
<tr>
<td></td>
<td>Losartan</td>
<td>4.3147</td>
<td>.000 HS</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>4.3147</td>
<td>.000 HS</td>
</tr>
</tbody>
</table>

** HS: Highly significant p<0.001
For salivary zinc the mean and standard deviation of patient on Amlodipine and Losartan treatment and for the control subjects showed in Table 4. The statistical analysis using ANOVA test demonstrated that salivary Zinc showed significant differences \((p<0.001)\) between the control group and the two study groups. Continuing analysis with \((LSD)\) test showed that the mean of salivary zinc in patients on Amlodipine and in patients on Losartan was significantly lower compared to control group \((p<0.001)\), while no significant difference between the mean of salivary zinc in patients on Amlodipine and on Losartan \((p>0.05)\) (Table 5).

**Table (4):** The mean and standard deviations of salivary Zinc in study groups with ANOVA

<table>
<thead>
<tr>
<th>Group</th>
<th>Zn μg/dl</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amlodipine</td>
<td>Mean 3.29</td>
<td>0.71</td>
</tr>
<tr>
<td>Losartan</td>
<td>Mean 3.45</td>
<td>0.75</td>
</tr>
<tr>
<td>Control</td>
<td>Mean 5.93</td>
<td>1.03</td>
</tr>
</tbody>
</table>

P-value .000

HS:-Highly significant at \(p<0.001\)

**Table (5):** Multiple comparison of Zinc using LSD among the study groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Subgroups</th>
<th>Subgroups</th>
<th>Std. Error</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>Amlodipine</td>
<td>Losartan</td>
<td>0.2191</td>
<td>0.476</td>
</tr>
<tr>
<td></td>
<td>Amlodipine</td>
<td>Control</td>
<td>0.2191</td>
<td>0.000 HS</td>
</tr>
<tr>
<td></td>
<td>Losartan</td>
<td>control</td>
<td>0.2191</td>
<td>0.000 HS</td>
</tr>
</tbody>
</table>

HS:-Highly significant at \(p<0.001\)

**Discussion**

Hypertension (HTN or HT), also known as high blood pressure, is define as a long-term medical condition in which arterial blood pressure is elevated persistently.

Sweet detection thresholds of sucrose (sweetness) of patients on Amlodipine and those on Losartan treatment were significantly higher than that of control subjects. Salt detection threshold in patients on Amlodipine and on Losartan treatment which were significantly higher than that of control subjects. For the sour detection threshold, it was lower in patients on Amlodipine and Losartan treatments than the control subjects but it did not reach the significant level. For the bitter detection thresholds it showed no significant differences between the study groups. This result was agree with Tsuruoka et al., 2005, who found that Losartan induced taste disturbances and they found that the taste disturbance by the Losartan and Perindopril (ACI-groups) medications at the dosages used was similar in quality and quantity. But disagree with Tsuruoka et al., (2005) who stated that Losartan-induced taste disturbances appear to be larger for “bitterness” and “sourness” than “salt” and “sweetness”. It has been hypothesized that a prominent underlying pharmacological mechanism, may explain taste disorders as a class effect of Angiotensin receptor blocker (ARBs). Taste receptors are seven-transmembrane domain G protein-coupled receptors. Angiotensin II receptors belong to the same type of receptor. The sweet and bitter receptors on taste cells are coupled with G-proteins, where G-protein coupling and uncoupling results in taste on and off, respectively ARBs are secreted into saliva, binding receptors of taste and thereby distorting the tastes of sweet and bitter. Salt and sour tastes may be disrupted by ARBs ion channels plugging or obstructing (salt taste via amiloride-sensitive epithelial Na channels, and sour taste via amiloride-sensitive epithelial Na channels and H+-activated cation channels) found on taste cells. This result was disagree with Kim et al., (2017) who reported that salt-taste thresholds did not significantly differ between the control and hypertension groups.

The result showed that salivary Zinc of patients on Amlodipine and for patients on Losartan treatment were highly significantly decreased than that of control subjects. This result agree with (Korean et al., 2005) who found that Losartan treatment result in Zn depletion, mediated by an increase in urinary Zinc excretion.
This result was also agree with (Ifor, 1989) who found that there is a decrease in the level of salivary Zinc level in hypertensive patient\textsuperscript{22}.

**Conclusions**

Amlodipine and Losartan can affect taste detection thresholds of sweet, salt in such a way that they were detected taste with high concentrations of taste solutions, but sour and bitter taste was not affected. And Zinc in saliva was decreased significantly by treatment with these medication.

**Conflict of Interest** We declare that we have no conflicts of interest.

**Human and Animal Rights** All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional research committee.

**Informed consent** Informed consent was obtained from all individual patients ,including in this study.

**Source of funding:** Self funding

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Analysis of Relationship between Work Attitudes and Repetitive Activities with Subjective Complaints on Musculoskeletal Disorder in Circular Loom Division workers PT. Kerta Rajasa Raya Sidoarjo Indonesia

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Abstract

The Musculoskeletal System Disorders (MSDs) are one of the most common occupational diseases experienced by workers. This disorder is influenced by several factors, such as repetitive activity factor and unnatural work attitude factor. The purpose of this study was to analyze the strong relationship between work attitude and repetitive activity with subjective complaints of musculoskeletal disorder in the circular loom division of PT. Kerta Rajasa Raya Sidoarjo, Indonesia.

This study used observational techniques with cross-sectional design. Respondents in this study were workers in the circular loom division of PT. Kerta Rajasa Raya, Sidoarjo, that consisted of 14 people based on the researchers’ inclusion criteria. The data were obtained from the results of questionnaires and field observations. Work attitude data were analyzed using OWAS; MSDs subjective complaint data were investigated using NBM checklist sheets; and repetitive activity data were analyzed based on the results of questionnaires, interviews, and observations.

The results showed that the majority of respondents have subjective complaints of musculoskeletal disorder with high severity. The work attitude variable has a very strong correlation with subjective complaints of MSDs, and repetitive activity variable has a strong correlation with subjective complaints of MSDs.

The company are advised to provide training related to safe lifting method, provide information related to MSDs in the form of posters or providing specific material. Companies are also advised to add the expedition personnel to reduce the repetition of lifting activities.

Keywords: Subjective complaints of MSDs, Work Attitudes, and Repetitive Activities

Introduction

The Musculoskeletal System Disorders are one of the most common occupational diseases experienced by workers. According to Tarwaka, Musculoskeletal disorder is a complaint in the part of the skeletal muscle that is felt by a person ranging from very mild to very painful complaints(¹). MSDs are the biggest cause of absence in almost all parts of Europe(²). According to the Labor Force Survey (LFS), the prevalence of musculoskeletal cases (MSDs) in the year 2016/2017 amounted to 507,000(³).

NIOSH worker health chartbook of musculoskeletal cases (MSDs) shows that manufacturing industry ranks second after the service industry(⁴). The manufacturing industry is an industry that converts raw materials into a
product. In the manufacturing industry, there are many manual handling activities that can cause the emergence of MSDs subjective complaints. Evadarianto found that the majority of manual handling workers as much as 73.34% in the rolling mill section experienced MSDs complaints\(^5\). Peter Vi in Tarwaka explained that the emergence of MSDs subjective complaints are influenced by several factors such as excessive muscle stretching, repetitive activity, unnatural work attitudes; secondary factors, the combination causes and individual factors\(^1\).

PT. Kerta Rajasa Raya Sidoarjo, Indonesia, is one of the manufacturing industries concerned in producing packaging that still has a lot of manual handling activities such as lifting, pushing, moving, or pulling heavy loads. One division with the most lifting activities is the circular loom division. Workers of the circular loom division regularly lift as much as 40-160 baskets per day. The weight of each basket is 17 kg. In addition, the work attitudes in lifting activities are often unnatural.

Based on the explanation, it means that workers who carry out manual handling activities have the potential to experience MSDs subjective complaints. Therefore the researcher is interested in conducting research related to the analysis of the strong relationship between work attitudes and repetitive activities with subjective complaints of musculoskeletal disorder in the circular loom division workers of PT. Kerta Rajasa Raya Sidoarjo, Indonesia.

The purpose of this study is to analyze the strong relationship between work attitude and repetitive activity with subjective complaints of musculoskeletal disorder in circular loom division workers of PT. Kerta Rajasa Raya Sidoarjo, Indonesia.

**Material and Method**

The type of research was observational with a cross-sectional approach. The study population is 14 workers, with the inclusion criteria of workers aged \( \geq 35 \) years, doing manual handling activities, and do not have a medical history of back pain, pain in the waist, sprains, and HNP (Hernia Nucleus Pulposus). The sample in this study is the total population.

Research variables are work attitudes, repetitive activities and subjective complaints of MSDs. The instruments used in the study are questionnaires, OWAS observation sheet (Ovako Working-Postur Analysis System), and NBM (Nordic Body Map) checklist sheets.

Analysis of the data used in this study is an analysis of the contingency coefficient for nominal scale data and correlation analysis of Spearman’s rho’s for ordinal scale data. The relationship between the dependent and independent variables will be classified according to Sarwono (2006)\(^6\):

<table>
<thead>
<tr>
<th>Contingency Value</th>
<th>Level of Relationship</th>
</tr>
</thead>
<tbody>
<tr>
<td>(0)</td>
<td>There Is No Correlation Between The Two Variables</td>
</tr>
<tr>
<td>(&gt;0,00 ) – (0,25)</td>
<td>Very Weak Correlation</td>
</tr>
<tr>
<td>(&gt;0,26 ) – (0,50)</td>
<td>Medium Correlation</td>
</tr>
<tr>
<td>(&gt;0,51 ) – (0,75)</td>
<td>Strong Correlation</td>
</tr>
<tr>
<td>(&gt;0,75 ) – (0,99)</td>
<td>Very Strong Correlation</td>
</tr>
<tr>
<td>(1)</td>
<td>Perfect Correlation</td>
</tr>
</tbody>
</table>

**Findings**

The circular loom division has three job sections, namely the expedition section which lifts a basket weighing 17 kg as much as 100-160 times, then the operator section of the circular loom that must lift and move the basket weighing 17 kg which is done 30-50 times in a working day, and the maintenance section of the equipment that is responsible for checking and repairing the machine.

**Table 2. Distribution of Frequency of**

<table>
<thead>
<tr>
<th>Severity of MSDs Subjective Complaints to Workers in the Circular Loom Division of PT Kerta Rajasa Raya Sidoarjo in 2019</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSDs Complaints</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Medium</td>
</tr>
<tr>
<td>High</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

The data above shows that all respondents experienced MSDs subjective complaints. As many as 57.1% experienced musculoskeletal subjective complaints with a high category and 42.9% of...
respondents in the medium category.

Table 3. Frequency Distribution of Risk level of work attitude and repetitive activity in Workers of the Circular Loom Division of PT Kerta Rajasa Raya Sidoarjo in 2019

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>n</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>work attitude</td>
<td>Medium</td>
<td>3</td>
<td>21.4</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>4</td>
<td>28.6</td>
</tr>
<tr>
<td></td>
<td>Very High</td>
<td>7</td>
<td>50.0</td>
</tr>
<tr>
<td>repetitive activity</td>
<td>10 - &lt;40</td>
<td>6</td>
<td>42.9</td>
</tr>
<tr>
<td></td>
<td>40 - &lt;200</td>
<td>8</td>
<td>57.1</td>
</tr>
</tbody>
</table>

Based on table 3, it was found that 50.0% of workers in the circular loom division had a very high-risk work attitude. The most repetitive activities carried out are 40 - ≤ 200 times every working day or 8 hours with a percentage of 57.1%.

The results of the analysis carried out between work attitude variables with MSDs subjective complaints give the Spearman Correlation value of 0.876. So, it can be concluded that there is a very strong correlation between work attitudes and subjective complaints of MSDs. The relationship direction is positive, which means that the relationship goes in the same direction or the higher the risk level of the workers’ attitude in doing their work, the higher the severity of subjective complaints of MSDs.

On analysis of repetitive activity variables, the closeness value of the relationship is 0.708 which is between the numbers 0.51-0.75. Thus, it can be interpreted that the relationship that occurs is strong. These values also provide information that the relationship between the variables of repetitive activity with subjective complaints of MSDs run in line for positive values. So that, the more repetitive lifting activities performed by workers in a day, then the severity of MSDs subjective complaints will also be higher.

Discussion

Musculoskeletal Disorders (MSDs) are a type of pain complaint that is felt in the skeletal muscle system. The pain is usually emerged in the joints, ligaments, or tendons. Complaints in the musculoskeletal system are usually in the form of chronic complaints that are often felt sometime after the worker does his activities and often leaves traces or residues that continue to be felt the next day. MSDs can be caused or aggravated by various risk factors in the workplace such as work attitudes, repetitive activity, vibration, and temperature (1).

Based on the results of research conducted at the circular loom division of PT. Kerta Rajasa Raya Sidoarjo, it was found that the majority of respondents experienced MSDs subjective complaints in the high category. All workers who experienced MSDs subjective complaints were operator workers and expedition workers who both carried out manual handling activities in the form of lifting baskets containing plastic thread rolls. The results of interviews conducted further found that the complaints felt by workers were spread almost throughout the body parts of workers. The location of complaints on the body felt by all workers respondents is the left and right shoulder, left and right upper arm, left and right forearm, and both legs.

Based on the results of the research and data processing of attitude variables with the OWAS method, it is known that all expedition workers have very high category of work attitudes whereas for the operator section 3 people have a medium risk, 4 people have a high risk, and 4 people have a very high risk. This is because the work of lifting in the expedition section is carried out with the body bent and twisted aside forming flexion. Moreover, the position of the hand when taking the basket is above shoulder height with a weight of 17 kg. So that, the value obtained by all expeditions is 4 (very high). This is different from the 3 operator workers who have a medium work attitude, even though the burden to be lifted is the same yet the lifting method is different. As many as 3 operators who have medium category of work attitudes are carrying out lifting activities without twisting their bodies so that for the back side get to point 2. Besides, the position of the two arms of the operator is also below the shoulder height so that his working attitude is in the medium category.

The results of the analysis get a very strong relationship between work attitude variables and MSDs subjective complaints experienced by workers. Therefore, the higher the risk of work attitude, MSDs subjective complaints will also increase. The results of interviews and observations were made found that all workers did not know about MSDs and workattitudes
matters. This was due to the education of workers who were mostly only up to high school. In addition, workers had never received information regarding good and correct work attitudes when doing work from the company. This research is in line with the research conducted by Purwosusilo (2015) which states that there is a significant relationship between work attitudes and subjective complaints of MSDs in traditional sand miners in Srumbug district, Magelang regency.(7).

Repetitive activity or repetitive movement is an activity carried out continuously(1). In the circular loom division of PT. Kerta Rajasa Raya Sidoarjo, the effect of repetitive activity is extremely felt in the expedition section. This is due to the expedition workers who are on average lifting 100-160 baskets daily. Expedition workers complain of pain in the shoulder, upper arm, forearm, calf, leg, and waist. The expedition section also did a basket arrangement on pallet truck as 5 tall stacks with a twisting body position that caused complaints on the workers’ waist.

Muscles complaints can occur when the muscles receiving pressure due to lifting the load continuously without obtaining sufficient relaxation time(1). This theory is in line with the results of research which state that there is a strong correlation between repetitive activity variables and MSDs subjective complaints. hence, the higher number of repetitions carried out, the subjective complaints MSDs will also increase. This study is not in line with the research conducted by Purwosusilo (2015) in traditional sand miners in Srumbug district, Magelang regency, which states that there is no relationship between the frequency of swinging with complaints on upper limbs; or in other words, there is no relationship between repetitive activity and musculoskeletal disorders.(7)

Conclusion

Based on the explanation above, it can be concluded that:

1. The majority of respondents have subjective complaints of musculoskeletal disorder with high severity.

2. There is a very strong relationship between work attitude variables with subjective complaints of musculoskeletal disorder.

3. There is a strong relationship between repetitive activity variables with subjective complaints of musculoskeletal disorder.

Recommendation

1. The company is advised to provide training regarding how to lift in the right position or attitude to reduce the risk of unnatural work attitudes.

2. The company is advised to provide information in the form of posters or work instruction procedures as a workers reminder related to the dangers of MSDs and safe lifting method to reduce the level of MSDs subjective complaints and risk of unnatural work attitude.

3. The company is advised to increase the number of expedition personnel by assisting operator section to reduce repetitive movements by expedition workers.

Conflict of Interest: All authors have no conflicts of interest to declare.

Source of Funding: This is an article “Analisis Faktor Penyebab Keluhan Subjektif Musculoskeletal Disorder (MSDs) ( pada Pekerja Shift Pagi Divisi Circular Loom PT. Kerta Rajasa Raya)” of Occupational Health and Safety Department that was supported by Faculty of Public Health, Airlangga University.

Ethical Clearance: The study was approved by the institutional Ethical Board of the Public Health, Airlangga University.

References


Application of Infection Control Rules by Iraqi Orthodontists

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1College of Dentistry/ Al –Iraqia University/ Iraq, 2Baghdad University\ Health Center

Abstract
Orthodontics represent an important dental speciality which deals different situations of patients malocclusion, they use many kinds of sharps and cutting instruments, making them liable to different types of infections which could be transmitted from either the patients or the instruments if they don’t follow the infection control principles. The data were collected by answering a questionnaire by 101 orthodontists, the results showed different responses of the participants to the variables used by the questionnaire leading to a conclusion that more efforts are needed to improve the application of infection control guidelines between Iraqi orthodontists.

Keywords: orthodontics, infection control, health, patients; infection.

Introduction
Orthodontists facing various types of microorganisms during practicing dentistry by contaminated instruments, inhalation of aerosols or through percutaneous injuries with different wires such as ligature or arch wires also banding and bonding materials and other sharp instruments(1).

Studies found that Orthodontists have the second highest occurrence among dental workers concerning hepatitis—B infections (2). People receiving treatment in dental clinic could be undetected hepatitis-B carriers and patients secreting herpes simplex viruses in saliva may be asymptomatic, those patients have the potential for transmitting diseases. Diseases such as hepatitis-B, HIV and tuberculosis have long incubation period and therefore, it is very difficult to detect the origin of such infections to the dental workers and other patients (3).

Before beginning with work the orthodontist should be clear about his or her goals in infection control criteria and it is mandatory to apply the most progressed method of disinfection and sterilization to get good results(4).

Sterilization kills all types of microorganisms including viruses, bacterial and mycotic spores.

Disinfection is the technique used for destruction or inhabition of the most pathogenic microorganisms and inactivating some viruses, therefore, reduction of microbial contamination to a safety level(5).

Hepatitis B virus can be spreaded by as little as 0.0004 ml blood while HIV by 0.1 ml blood(6). 1 ml of gingival crevicular fluid contains 150 billion microorganisms and 6 billion microorganisms can be found in 1 ml of saliva(7).

Many studies in literature reveals that emphasize on the effect of sterilization in orthodontics practice however there is no comprehensive research that evaluate the compliance of Iraqi orthodontist to infection control procedures.

Aim of the Study
In this study we will evaluate sterilization and disinfection methods employed in orthodontic practice in Iraq.

Material and Method
In the present study, data collection gained by an 17 items questionnaire was delivered to a total of 101 Iraqi orthodontists / general practitioners(GP) (who attended intensive orthodontic course), these question covered some infection control guide line(8).

Statistical analysis was performed by using SPSS version which includes descriptive statistic (frequency
and percent chi square test was used to find association between related variables, \( P>0.05 \) was considered significant.

The questionnaire deals with the following variables:
- Educational degree
- Place of work.
- Daily patient volume
- The way in performance of instruments cleaning
- Sterilization devices used
- Soaking instruments in disinfectant solution.
- Packaging method of instruments to be sterilized.
- Method used to sterile hand piece, hand instruments and orthodontic pliers.
- Does the practitioner sterilize molar bands after purchase?
- Sterilization of molar bands after trail inside patient mouth.
- Disposal of brackets, bands and arch wires removed from the patients
- Recycling brackets or other orthodontic materials.
- Disinfectios of the impressions and orthodontic appliances delivered to the lab.
- Place of sharp objects disposal container.
- Type of gloves used during cleaning instruments, and environmental cleaning.
- Hepatitis B vaccination

### Result

The result of this study shown in the frequency table explained as the following: table 1 describes the percent of GP and specialist who are alligated to the questioner, (22.8\% and 77.2\%)for GP & specialist respectivlly.

Table 2 showed that (45.5\%, 38\% and 15.8 \%) of the total participate work in private clinic, hospital and at university clinic, those participate have daily patients volume explained in table 3.

Table 4 show (57.4\%) of orthodontist who use manual cleaning significantly higher than ultrasonic cleaner (42.6 \%), while highly significant (87\%) with autoclave rather than oven and glass bead sterilizer, also highly significant regarding presoaked instrument and wrapping the instrument, while sterilization of dental hand piece in autoclave (41.6\%) was significantly lower than wiping the outer surfaces with (57.4\%).

Table 5 show highly significant rate of sterilize pliers (80.2\%) than dry heat, while non-significant sterilize band after purchase in comper with highly significant (67.3\%) for band after trial in patient’s mouth. The disposing of band, bracket and arch wire was highly significant with (49.5\%) regarding waste basket.

Table 6 show high rate of significantly for orthodontist who don’t recycled bracket while non-significant difference between orthodontist who disinfect impression or appliances. The rate for placing sharp bins at clinic was significant (74.3\%) and (21.8\%) for placing sharp bins in sterilization room. Highly significant rate for orthodontists who use examination gloves during cleaning of the instrument, and how recive hepatitis B vaccine.

<table>
<thead>
<tr>
<th>Table 1 Gp or Specialist</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>GP</td>
<td>23</td>
<td>22.8</td>
</tr>
<tr>
<td>Specialist</td>
<td>78</td>
<td>77.2</td>
</tr>
<tr>
<td>Total</td>
<td>101</td>
<td>100.0</td>
</tr>
</tbody>
</table>
### Table 2: Working Place

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Private dental clinic</td>
<td>45.5</td>
</tr>
<tr>
<td>Specielized dental centers / state hospital</td>
<td>38.6</td>
</tr>
<tr>
<td>University clinic</td>
<td>15.8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

### Table 3: Number of Patient

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-5</td>
<td>39.6</td>
</tr>
<tr>
<td>6-10</td>
<td>17.8</td>
</tr>
<tr>
<td>11-15</td>
<td>14.9</td>
</tr>
<tr>
<td>16-20</td>
<td>14.9</td>
</tr>
<tr>
<td>&gt; 20</td>
<td>12.9</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>99.0</strong></td>
</tr>
</tbody>
</table>

### Table 4: Percentage of Cleaning and Sterilization of the Instrument

<table>
<thead>
<tr>
<th>Question</th>
<th>Choice</th>
<th>Frequency</th>
<th>%</th>
<th>P-value¥</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cleaning performance</td>
<td>Manually</td>
<td>58</td>
<td>57.4</td>
<td>0.033</td>
</tr>
<tr>
<td></td>
<td>Mechanically (Ultrasonic cleaner)</td>
<td>43</td>
<td>42.6</td>
<td></td>
</tr>
<tr>
<td>Sterilization devices used</td>
<td>Autoclave</td>
<td>88</td>
<td>87.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dry heat (oven)</td>
<td>12</td>
<td>11.9</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Glass bead sterilizer</td>
<td>0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>none</td>
<td>1</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>The instruments are presoaked in disinfectant solution</td>
<td>Yes</td>
<td>86</td>
<td>85.1</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>15</td>
<td>14.9</td>
<td></td>
</tr>
</tbody>
</table>
### In Instruments Packing during sterilization

<table>
<thead>
<tr>
<th>Choice</th>
<th>Frequency</th>
<th>%</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wrpa (Pouching)</td>
<td>41</td>
<td>40.6</td>
<td>0.021</td>
</tr>
<tr>
<td>I do not pack</td>
<td>20</td>
<td>19.8</td>
<td></td>
</tr>
</tbody>
</table>

### In Sterilization of dental hand pieces

<table>
<thead>
<tr>
<th>Choice</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>In the autoclave</td>
<td>42</td>
<td>41.6</td>
</tr>
<tr>
<td>Wiping the outer surface with disinfection solution</td>
<td>58</td>
<td>57.4</td>
</tr>
</tbody>
</table>

### Table 5: percentage of sterilization of orthodontic pliers, bands and disposed method

<table>
<thead>
<tr>
<th>Question</th>
<th>Choice</th>
<th>Frequency</th>
<th>%</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterilization of hand instruments / or orthodontic pliers</td>
<td>Dry heat (oven)</td>
<td>12</td>
<td>11.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Autoclave</td>
<td>81</td>
<td>80.2</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Glass bead sterilizer</td>
<td>1</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wiping with a disinfectant solution</td>
<td>6</td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>Sterilization of molar bands after purchase</td>
<td>Yes</td>
<td>48</td>
<td>47.5</td>
<td>0.617</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>53</td>
<td>52.5</td>
<td></td>
</tr>
<tr>
<td>Sterilization of molar bands after check in the patient mouth</td>
<td>Dry heat (oven)</td>
<td>10</td>
<td>9.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Autoclave</td>
<td>68</td>
<td>67.3</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>glass bead sterilizer</td>
<td>2</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sitting in disinfectant solution</td>
<td>17</td>
<td>16.8</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Question</th>
<th>Choice</th>
<th>Frequency</th>
<th>%</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Where do you dispose the bands, brackets, and arch wires you remove from patient during or after treatment</td>
<td>Waste basket</td>
<td>50</td>
<td>49.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sharp bin</td>
<td>27</td>
<td>26.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Metal waste bin</td>
<td>6</td>
<td>5.9</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Infected waste bin</td>
<td>17</td>
<td>16.8</td>
<td></td>
</tr>
</tbody>
</table>
### Table 6: the response to different question related to infection control roles

<table>
<thead>
<tr>
<th>Question</th>
<th>Choice</th>
<th>Frequency</th>
<th>%</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recycled brackets or other orthodontic materials</td>
<td>Yes</td>
<td>9</td>
<td>8.9</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>91</td>
<td>90.1</td>
<td></td>
</tr>
<tr>
<td>Disinfection of impressions or other orthodontic appliances to be delivered to lab</td>
<td>Yes</td>
<td>48</td>
<td>47.5</td>
<td>0.670</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>51</td>
<td>50.5</td>
<td></td>
</tr>
<tr>
<td>Where do you place sharp bins at the clinic</td>
<td>In the sterilization room</td>
<td>22</td>
<td>21.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>at the clinic</td>
<td>75</td>
<td>74.3</td>
<td></td>
</tr>
<tr>
<td>Type of gloves used during cleaning of instruments and environmental cleaning</td>
<td>Examination gloves</td>
<td>97</td>
<td>96.0</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Kitchen - type gloves</td>
<td>3</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>Have you had hepatitis B vaccine</td>
<td>Yes</td>
<td>83</td>
<td>82.2</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>16</td>
<td>15.8</td>
<td></td>
</tr>
</tbody>
</table>

### Discussion

Spaulding system classifies instrument into three categories which are critical, semicritical and least critical\(^{(9)}\), the semicritical considered the most important one that should be highlighted in order to prevent disease transmission \(^{(10)}\). Orthodontic instruments, orthodontic supplies and accessories considered as the semicritical since they touch mucous membrane and non-intact skin \(^{(11,12)}\).

This study mainly depended on experience, place of work as well as, daily patient capacity of the GP and orthodontist. The sterilization process is important to orthodontists as well as, dentists, even though they do not perform surgical procedures \(^{(13-14)}\).

On the other hand Starnbach & Akçam \(^{(15-16)}\) indicate, sterilization is less abidance to orthodontists than dentists since they usually deal with children, with loss of time, money and the corrosion of orthodontic instruments in addition they did not deal substitute the word (with) by ( may lead to) deep tissues.\(^{(8)}\)

Professional agencies like Center of Disease Control (CDC) and Occupational Safety and Health Administration (OSHA), have a specific recommendations representing standard infection control which considered as keywords to be followed in order to prevent cross infection \(^{(17)}\).

Despite of these rules, this study showed that \((57.4\%)\) of participate orthodontists depends on the manual cleaning procedure more significantly than the ultra-sonic devises. This may be due to the lack of knowledge about advantage of ultra-sonic devises in the granting the proper removing of debris from the orthodontic instrument \(^{(18-19)}\), the manual cleaning is also an important step in ensuring the debris removal after mechanical cleaning and before autoclaving \(^{(20)}\).

In this study \((85.1\%)\) of participated presoaked the instrument in disinfected solution before starting the sterilization process. This prevents the dryness and adherence of bioburden to instruments that protect microorganism from sterilization \(^{(21)}\).

In addition to that it begins to dissolve organic debris and in some instances begin microbial kill. This solution should discard at least once a day \(^{(17)}\).

This study showed high percentage of participates who used autoclave whether used for orthodontic pliers, band purches and molar bands after trying in patient mouth \(^{(22)}\). This concurred with the significant findings of participates who pouch their instruments.
This could be explained by the high education level and interest in sterilization of orthodontists and according to recommendations of Iraqi Dental Association (IDA).

Although most of orthodontist (87%) using autoclave but the result showed that high percentage of them only wipes the hand piece which is not recommended as the hand piece represents a hallow instrument that may contain blood drops contamination inside the internal lumen and this can be only sterilize by using autoclave class B (23, 24-25-26). This could be the result of old believes that heat could ruin the hand piece leading to a financial lost.

As a matter of fact the hand piece sterilization is obligatory according to the CDC guidelines. This can be achieved either by providing clinics with an enough number of hand piece in order to match the number of patients who daily visit the clinics, or by applying advanced sterilization programs (27). Since hand pieces are available in the Iraqi market with an affordable cost.

Bracket, wire and bands represents a dangerous source since they are removed from oral cavity in which they had been contaminated with body fluid (saliva and blood) also wire’s end considered as a sharp end that may prick the orthodontist, so the sharp pin represent the better choice for disposing (28,29).

This study showed that (74.3%) of participant have sharp bin in their clinics, despite of the importance value of having it in the clinics, only (26.7%) who are really using it while (49.5%) of the participant use waste basket. This approved a week point in disposal.

This study showed a highly significant percentage (90.1%) of the participant who do not use recycle brackets and orthodontic materials since the process of recycling altars the mechanical and physical properties also it is nor granted that they are not contaminated(30,31) also, the brackets and orthodontic materials are available in the Iraqi market with a reasonable cost.

In this study, a non-significant value (50.5% -47.5%) have been shown between the participates who disinfected their impression or appliances to be delivered to an outer laboratory. Standers showed that all impression and model must be disinfected before delivering to the laboratory and vice versa (32). Now a day, this complicated issue has been solved by introducing the digital scan in the dental filed (33).

Kitchen gloves considered as a heavy duty gloves that protect the operator from accidental puncher by sharp dental tools and cross infection (17). Unfortunately, this study showed a high rating of (96%) who use examination gloves which considered a thinner and easy tearing gloves as compared to the kitchen gloves (8).

Dentists and assistances are always mandatory to be vaccinated against hepatitis B virus (34), this coincide with the result of this study which revealed the highly significant rating (82.2%) who had been vaccinated.

Conclusion

The result of this study reveals good behaviours by Iraqi orthodontist for most of infection control steps although some behaviors need to be improved following world wide infection control guide lines.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None.

Funding: Self-funding

References


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Mast cell, IL-1 Beta and IL-6 for Wound timing and Vitality in Forensic Practice

Mohammad Abdul Mohsin Jebur, Nabeel Ghazi Hashim, Salim R. Hamoudi Al-Obeidi

Abstract

the application of immunohistochemistry has opened a new era for examining the age of the wound by forensic specialists, The aim of our study was to illustrate the use of immunohistochemical marker of mast cells activation (mast cell tryptase “MCT”) as a reliable and promising factor of wounds vitality and to Identify the role of pro-inflammatory cytokines: IL 1 beta and IL 6 as parameters of wound age determination. The study was done in the Medico-Legal Directorate of Forensic Medicine (MLD) in Baghdad. The specimens were sera and skin tissues for immunohistochemistry examination, and staining procedures and immunohistochemistry (MCT) ELISA sandwich plate will measure the levels of interleukins. Total autopsy cases were 88 and divided into study group (SG) and control group (CG). We found that density of Mast cells in sample group was significantly high and higher infiltration that correlates with time passing in the study group as compared by other group in dermis of the sample and control lesions and positive relationship between levels of IL 1-beta & IL-6 level and time progress of wound..

Key words: forensic, pathology, wound, vitality, IL 1 beta, IL-6 level, Mast cells

Introduction

During the inflammatory phase, a variety of chemicals are released at the affected site, leading to the recruitment of inflammatory cells, such as neutrophils and plaques Forensic scientists, unlike general pathologists, tend to focus on chronic mapping of the appearance and disappearance of inflammatory cells or substances produced during the inflammatory process[1]. These phenomena - for example, the proportion of positive cells, the level of tissue fibrosis, the distance between inflammatory cells and the free vessels that affected by the degree of injury, which affect the accuracy of the determination of the age of the wound. It is therefore necessary to establish models with varying degrees of injury and to evaluate the parameters involved in wound healing to determine the time of injury[2].

Thus, MCs have been involved in causing many chronic allergic / inflammatory disorders, autoimmune diseases, and cancers. The contributions of MCs in these conditions are the subject of continuous assessment[3]. Acute direct events, the allergic process involves subsequent stages characterized by leukocytes infiltration and initiation of an acquired immune response, followed by a chronic phase involving persistent inflammation, tissue reformation and fibrosis. Thus, the role of MCs at these different stages has gained increasing importance. The dissociation of MCs, in addition to their well-established and widely studied role in IgE-mediated interactions, has been the focus of MCs research in the past decades. However, the determination of the functions of MCs may progress slowly due to difficulties in accessing these cells in vivo and the obstacles encountered when obtained through enzymatic dispersion of tissues or by culture of predators of MCs isolated from the bone marrow or cord or peripheral blood. The culture of predators of mast cells produces a small number of MCs that are often expensive and time-consuming and lead to the emergence of changing phenotypes due to cultural conditions[4].

Several studies reported high levels of IL-1β production by nuclear monocyte, platelet and nuclei cells from active colon lesions in IBD (inflammatory bowel disease) patients. Since IL-1RA levels are only moderately controlled in the colon in patients with IBD, the IL-1RA ratio to IL-1β decreases significantly,
enhancing intestinal inflammation\textsuperscript{[5]}. IL-1\(\beta\) is initially produced as zymogen, but pro-IL-1\(\beta\) does not contain any known functional properties. IL-1\(\beta\) copies are tightly regulated, stimulated by stimulation by TIR or IL-1R stimulation (the first signal required to produce IL-1\(\beta\)). As previously described, IL-1\(\beta\) precursors are bisection in its active form by caspase-1, after inflammatory activation with the NLR (NOD like receptor: NOD for nucleotide-binding domain, Lucien rich repeat containing receptors) pathway (signal 2) \textsuperscript{[6]}. The secretion mechanisms of IL-1\(\beta\) and IL-18 in extracellular space are unclear, but proposed pathways include exocytosis of secretory lysosomes, shedding of membrane micro-vesicles or exosomes, or transfer by membrane vectors, such as ASC (alanine serine cysteine amino-acids transport system) transporters\textsuperscript{[7]}.

IL-1\(\beta\) practices a wide range of systemic and local effects. Once in circulation, IL-1\(\beta\) can promote the synthesis of ring-2 oxidation enzymes (COX2) in the vascular network, which stimulates the production of prostaglandin E2 in the brain and fever-mediated. IL-1\(\beta\) also promotes the synthesis of acute phase proteins by liver cells, derives differentiation of macrophages, differentiation of neutrophils and mobilization in bone marrow. Furthermore, IL-1\(\beta\) promotes the recruitment of immune cells to inflammatory sites, by stimulating the expression of adhesion molecules and chemical attractors via endothelial cells\textsuperscript{[8]}.

On the other hand, given the IL-6 behavior of restoring the host to the parity state, it is clear that IL-6 acts to control the response of tissue inflammation. In chronic diseases, which are usually manifested by immune stress factors such as intracellular infection and chronic tumors, IL-6 acts not only as a catalyst for acute phase reactions but also as an important player in cellular immune responses to infected cells and mucosal- The inflammatory reaction is acute, resulting in the destruction of a harmful agent within a short period of time and in a localized area, stimulating an immune response.[8]

IL-6 not only stimulates acute phase reactions but also develops cellular immune responses, including end-stage B cell differentiation, immune globulin secretion and T-cell activation. The main key from acute inflammation to chronic inflammation is the recruitment of monsters to the inflammation area. IL-6 is important for the transition between acute and chronic inflammation. IL-6 plays a somewhat unexpected role in recruiting white cells in vivo. The IL-6 and sIL-6Ra compounds can activate endothelial cells to secrete IL-8 protein and MCP -1, and urge the expression of adhesion molecules\textsuperscript{[9]}.

The aim of the study is to illustrate the use of immunohistochemical marker of mast cells activation (mast cell tryptase “MCT”) as a reliable and promising factor of wounds vitality and to identify the role of pro-inflammatory cytokines: IL 1 beta and IL 6 as parameters of wound age determination.

**Methodology**

The study was done in the Medico-Legal Directorate of Forensic Medicine (MLD) in Baghdad. Lacerated skin wounds with a known timing since injury less than 12 hours, regarding time of autopsy all cases were underwent autopsy as soon as possible within a maximum time of 3 hours since arrival to mortality. Only frank lacerated non-contaminated skin wounds were taken in account and the autopsy was done immediately on arrival to MLD. Total autopsy cases were 88 and divided into study group (SG) and control group (CG) [stab-wounds injuries with immediate death]. The specimens are sera and skin tissues for MCT immunohistochemistry.

ELISA sandwich plate will measure the levels of interleukins and specimens for histopathology will be taken from the periphery of wounds for SG and an intact healthy skin from same cadaver (internal control) and some tissue specimens from deaths due immediate death by bullet injury as (external control), stored in 10% formalin for staining procedures and immunohistochemistry (MCT) and the results was analyzed statistically.

Strict exclusion criteria for cases of study include mixed wounds, wounds with unknown timing since injury, contaminated lacerated wounds, wounds with expected timing less than 12 hours, firearm deaths, cases with a known documented history of chronic illnesses, age less than 15 and more than 35 years, pregnant, decomposed cadavers (delayed autopsy), major medical interventions like surgery.

The Quantikine® Human IL-1\(\beta\)/IL-1F2 Immunoassay is a 3.5-4.5 hour solid phase ELISA designed to measure human IL-1\(\beta\) in cell culture supernates, serum, and plasma. The Quantikine® Human IL-6 Immunoassay is a 4.5 hour solid phase. Both contain E. coli-expressed recombinant human IL-1\(\beta\) and IL 6...
and antibodies raised against the recombinant factors.

TB staining was used for presence of “Mast cells” and MCT immunohistochemistry by Elabsceince® is the preferable way for detecting mast cells activation.

Results

The number of wounds in the study sample was determined; most of the bodies had three wounds, 44% of the total study, where 6% of the cases had only one wound. The study group of 156 laceration skin wounds was classified into compression laceration (50%), grinding laceration (20.5%), cut laceration (6.5%), tearing (15.3%) and crush injuries (7.7%).

Mast cells by toluidine blue stain

Mast cells are found in connective tissue and the cytoplasm contains (heterogeneous) granules consisting of heparin and histamine. Toluidine blue stains mast cells with red-purple color (metachromatic staining) and blue background (orthochromatic staining). Metachromasia and tissue color staining elements differ according to the features of the dye solution due to the pH, dye concentration and temperature of the underlying dye. Blue or purple dyes will show a red shift while red dyes will show a yellow shift with contrasting tissue elements.

Depending on the number of mast cells stained by toluidine blue, three classes of were estimated, from 2 to 5 cells on 10 HPF the number of mast cells stained was regarded normal finding, from 6-8 cells on 10 HPF (+1) and from 9-10 cells on 10 HPF and (+2) and > 10 cells on 10 HPF (+3) The specimens included in the study did not have any histologic abnormality. Using light microscopy, all the mast cells (MC) in the specimens (however fixed) had meta-chromatically stained purple with toluidine blue. The nuclei of all the cells were round or oval. MC were scattered in the dermis, especially along the blood vessels and in the peri-glandular stroma. In the dermis of the Sample and control lesions, Mast cell density in the Sample group was significantly higher (P<0.001) when compared with the other group.

Table 1: Distribution of mast cells

Immunohistochemistry (Mast cells tryptase) for both samples and control

Immunohistochemistry of Mast Cell Tryptase results were recognized and classified into 4 classes according to the mast cells in both control and samples through the period of 6 hours after injury. Tryptase immune reactivity was detected in all of the cells stained by fluorescent avidin fixed either with Bouin or Carnoy’s fluid. Some MC, in the dermis of all the examined groups, was immune stained for chymase upon fixation with Carnoy’s fluid.

Table 2 correlation between test group and control group number of mast cells

ELISA levels of IL-1 beta and IL-6 in the human model

IL-1 beta standard of the kit measures at 3.9 pg/ml and minimum detectable dose (MDD) of human IL-1 beta is less than 1 pg/ml. The levels of IL-1 beta in the human model shows a significant decrease in relation to the time of the test (1 to 6 hours AMI), as showed in table 2 that the levels of IL-1 beta in the human model samples shows a maximum mean difference of 40.727 at 1 hour of AMI while the control group mean difference was 284.584. The mean difference decreased by the increasing of time after AMI to reach 3.49 after six hours. The study results suggested a reverse relationship between AMI time and the levels of IL-1 beta in the human model.

Table 3: The levels of IL-1 beta in the human model

H1, after one hour AMI H4, after four hours AMI
H2, after two hours AMI H5, after five hours AMI
H3, after three hours AMI H6, after six hours AMI

IL-6:

IL-6 standard of the kit measures at 3.13 pg/ml and the MDD of human IL-6 is typically less than 0.70 pg/ml. The IL-6 levels show increase with the time of AMI progress. The control samples show mean difference of .57436. The sample group shows maximum mean difference of IL-6 level of 353 at five hours of AMI.
Table 4: The levels of IL-6 in the human model

<table>
<thead>
<tr>
<th>Test Value = 0</th>
<th>t</th>
<th>df</th>
<th>Sig.</th>
<th>Mean Difference</th>
<th>95% Confidence Interval of the Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
</tr>
<tr>
<td>c</td>
<td>5.980</td>
<td>10</td>
<td>.000</td>
<td>.57436</td>
<td>.3603</td>
</tr>
<tr>
<td>h1</td>
<td>1.127</td>
<td>10</td>
<td>.286</td>
<td>269.933</td>
<td>263.73</td>
</tr>
<tr>
<td>h2</td>
<td>15.359</td>
<td>7</td>
<td>.000</td>
<td>2.16250</td>
<td>1.8296</td>
</tr>
<tr>
<td>h3</td>
<td>13.272</td>
<td>9</td>
<td>.000</td>
<td>1.40290</td>
<td>1.1638</td>
</tr>
<tr>
<td>h4</td>
<td>9.805</td>
<td>5</td>
<td>.000</td>
<td>1.89883</td>
<td>1.4010</td>
</tr>
<tr>
<td>h5</td>
<td>1.006</td>
<td>3</td>
<td>.389</td>
<td>353.03625</td>
<td>763.9650</td>
</tr>
<tr>
<td>h6</td>
<td>6.926</td>
<td>4</td>
<td>.002</td>
<td>.63980</td>
<td>.3833</td>
</tr>
</tbody>
</table>

H1, after one hour AMI  H4, after four hours AMI  c, control

H2, after two hours AMI  H5, after five hours AMI

H3, after three hours AMI  H6, after six hours AMI

Discussion

In this study we found that density of Mast cells in sample group was significantly high (P<0.001) as compared by other group in dermis of the sample and control lesions. This result goes with the results found by Bonilli et al. [10] in the contrary to[11] who noted no significantly different mast cells’ number of in normal cutaneous tissue & those in wounds of the sampling which could be explained partly by different techniques & morph-metrical methodology.

In this study we found higher infiltration with mast cells that correlates with time passing in the study group which agrees with the results found by [10] who utilized anti-trypase & chymase antibodies or avidin to assess density of mast cells in skin wounds through immunofluoresences And found that the MC number in the dermis showed progressive increase within little hours after injury (top at 1 to 3 hours) Matching results was found by [12] who noted a powerful extra-expression of trypase located in interstitium.

Levels of IL-1 beta in the human model samples show a maximum mean difference of 40.727 at 1 hour of AMI while in control group, the mean difference was 284.584. The mean difference decreased by the increasing of time after AMI to reach 3.49 after six hours .The study results suggested a reverse relationship between AMI time and the levels of IL-1 beta in the human model. This results matches with [13] who found IL-1β, expressed in normal human skin modified in a significant way in vital injuries in epidermal strata, sub-epidermal cellular, vascular and glandular (sweat) and had promoted expression after fifteen and twenty minutes at early increase of reactivity of the epidermis & following thirty to sixty minutes, marked expression was noted, remained many hours & later decreased to a base level again. He commented that it can help as a beneficial method to estimate vitality and aging of wounds, especially during early post-traumatic period before the leukocytic reactions. On another hand this is against what was found by [14] who stated that no wound groups showed a high increase in IL-1β level in comparison to control group. The difference was not significant from statistical view.

In our study the IL-6 levels shows increase with the time of AMI progress which matches with what was found by [15] and the same results was obtained by [16]
Conclusion

Mast cells by toluidine blue stain density in the sample group was significantly higher when compared with the other group in the dermis of the sample and control lesions. The same was shown by Immunohistochemistry (Mast cells tryptase) as there were higher infiltration with mast cells that correlates with time passing in the study group. Regarding ELISA levels of IL-1 beta and IL-6 in the human model the study found a reverse relationship between AMI time and the levels of IL-1 beta in the human model while the IL-6 levels shows increase with the time of AMI progress.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

References

Association between Genetic Polymorphism of 5-HTTLPR, and SGOT, SGPT and Catalase with Alcoholism of Iraqi People

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Abstract

A case-control study on the relationship of 5-HTTLPR gene polymorphism with alcohol abuse among Iraqi individuals was conducted during period from December 2018 to June 2019. DNA was extracted from the blood samples of the study subjects. The genetic polymorphism analysis was conducted by Restriction Fragment Length Polymorphism (RFLP-PCR) for SLC6A4 gene. The results raveled that the odd ratio for the LL genotype was 1.697 indicating that homo mutant genotype were at higher risk for alcoholism than the wild type SS. This result showed that L allele frequency was (0.78) in alcoholism group and (0.53) in control group whereas S allele frequency was (0.22) in the alcoholism group and (0.47) in the control group. So, the results showed there was a significant difference in allele frequencies of SLC6A4 gene between case and control (P<0.01; OR=1.697; 95% CI=0.86-1.64). There was a significant positive correlation between the SGOT activity and the genotype of 5-HTTLPR (SS, SL, LL). Additionally, there was a significantly increased level of liver enzymes (GOT and GPT) and catalase in serum samples of alcoholic males compared with non-alcoholic males.

Key Words: SLC6A4, 5-HTTLPR, SGOT, SGPT, catalase.

Introduction

Alcohol dependence and alcohol abuse are chronic disorders comprising a wide range of clinical symptoms. Given that drinking behaviors are jointly determined by genetic and environmental risk factors, alcohol consumption and alcohol use disorders are appropriate phenotypes for investigating gene-environment interactions (1). Alcohol consumption is a common, complex trait, and heavy alcohol use increases the risk of alcohol use disorders, and is recognized as, a problematic global problem threatening both individual development, family life and social life of a person (2). There is an evidence of a causal relationship between alcohol and at least 200 diseases including gastritis, pancreatitis, cardiovascular disease, liver cirrhosis, hepato cellular carcinoma, and gastric cancer (3). Ethanol is not stored in the body after ingestion because it is fully ingested oxidized during metabolism in the liver (4). The rate of ethanol metabolism determines the concentration of ethanol and its metabolite acetaldehyde in the different tissues, which in turn influences the effects of ethanol consumption on liver and the other organs. Serotonin is a signaling molecule with a widespread effect in the CNS, and has a very important role in different aspects of mammalian life, like food intake, emotion, mood, respiration, pain sensitivity, cardiovascular regulation, sexual behavior, learning and memory, circadian rhythm, sensorimotor activity, and cognition (5).

The human serotonin transporter (SETR) is a monoamine transporter protein, encoded by a single gene (SLC6A4, solute carrier family 6, member 4) located on the long arm of chromosome 17 (17q11.2). The serotonin transporter (5-HTT) is an important protein responsible for the active transport of serotonin into neurons, enterochromaffin cells and platelets (6). Twin studies have shown alcohol dependence (AD) to have a heritability of ~50–60% (7, 8). Among the genetic components, many of the genes that may contribute to the risk of alcohol phenotypes encode components of the dopamine, serotonin (5-HT), and acetylcholine neurotransmitter systems.
Material and Method

Study Subjects

A case-control study on the relationship of 5-HTTLPR polymorphism with alcohol abuse among Iraqi individuals was conducted during period from December 2018 to June 2019. Fifty blood samples of alcoholic male Iraqi people (with a history of alcohol abuse for more than seven years) were collected from the Institute of forensic medicine, Al-Yarmouk Teaching Hospital, Shaikh Zayed Hospital, and Ibn-Rushed Teaching Hospital/Baghdad, Iraq. The study subjects were men of ages mean of 35.04 years ± 10.89 SD. Additionally, 50 samples were collected from non-alcoholic subjects, as control group, with mean age of 34.30 years ± 10.86.

Blood Sampling

Five milliliters of blood were collected by vein puncture, two ml was put into EDTA tubes for molecular analysis and three ml put in separating gel tube, then was allowed to clot at room temperature for 30 minutes and then centrifuged at 2000 rpm/15 minutes. The sera were collected and stored at -20°C until analysis.

Enzymatic Assay

The liver enzymes (GOT and GPT) and catalase in serum samples of alcoholic and non-alcoholic males were measured by Reflontron/Germany in this study.

Genotyping of SLC6A4 Polymorphism

Preparation of Genomic DNA

The DNA from genome was prepared from blood samples (gSYNC™ DNA Extraction Kit) according to the instructions of the manufactured. Concentrations and purity of DNA were measured by Nano drop spectrophotometer (Apel/Germany).

PCR condition and Restriction Fragment Length Polymorphism (RFLP)

Two primers were selected (F-5’- GGC GTT GCC GCT CTG AAT GC -3 ) and (R-5’- GAG GGA CTG AGC TGG ACA ACC AC -3 ′) (9) to amplify fragments of (469 &512) bp for the detection of alleles. The specific designed primers were provided by AccuOligo/ Bioneer/ Korea. The PCR reaction was performed in a total volume 20 µl containing 10 p mole/µl of each primer, 1x Master mix(AccuPower® ProFiTaq PCR PreMix/ Bioneer/ Korea), and 0.15µg/µl genomic DNA. The reaction mixture was amplified in thermal cycler (Cleaver Scientific, UK). Initial denaturation was carried out at 95°C for 5 min and the target DNA was amplified in 40 cycles. Subsequently, each cycle consisted of denaturation at 95°C for 30 sec; followed by annealing at 63°C for 30 sec. Elongation was carried out at 72°C for 1 min. The final extension step was performed at 72°C for 10 min. Aliquots of products of amplified DNA were treated with MspI (restriction enzyme, SibEnzyme/ Russia). The PCR product (5-HTTLPR) was digested with restriction endonucleases in a total volume of 20µl containing 10 units of enzyme with buffers supplied by the manufacturer’s instructions. The amplified PCR products were checked for the expected size on 2% (w/v) agarose gel and visualized after staining with ethidium bromide under ultraviolet. A 100bp DNA molecular weight marker (BioNeer/Korea) was used to measure the weight of the fragments (10).

Biostatistical consideration

The (11) program was used to analyze the difference factors in study variables. Chi-square test was used to compare differences among percentage at P-values of 0.05 and 0.01 probability. Odd ratio and 95% CI were estimated.

Results and Discussion

The study subjects were men of ages mean of 35.04 years ± 10.89 SD. Additionally, 50 samples were collected from non-alcoholic subjects, as control group, with mean age of 34.30 years ± 10.86, table (1).

Table (1): Distribution of ages of alcoholic male among cases and control

<table>
<thead>
<tr>
<th>Age group (year)</th>
<th>Group</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>&lt; 30</td>
<td>16</td>
<td>(32.00%)</td>
<td>14 (28.00%)</td>
</tr>
<tr>
<td>30-40</td>
<td>20</td>
<td>(40.00%)</td>
<td>25 (50.00%)</td>
</tr>
<tr>
<td>&gt; 40</td>
<td>14</td>
<td>(28.00%)</td>
<td>11 (22.00%)</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td></td>
<td>50</td>
</tr>
</tbody>
</table>

Results listed in table (1) indicated that the most common age group of cases(alcohol consumption) was
of 30-40 years (40.00%) followed by the group less than 30 years (32.00%), and more than 40 years (28.00%).

**Blood Chemistry Analysis**

The results of testing for GPT and GOT in serum from the cases as compared to the controls are presented in figure (1: A, B).

![Figure 1: Comparison of liver enzymes; A (GOT) and B (GPT) in serum samples of alcoholic and non-alcoholic males](image1)

The results in the present study indicated that parameters measured (GPT and GOT), which are markers liver functions revealed significant differences between cases and controls, \((42.46 \pm 23.39 \text{ vs. } 19.89 \pm 11.31)\) \((48.69 \pm 21.31 \text{ vs. } 15.93 \pm 9.27)\) at \((P<0.01)\) for GPT and GOT, respectively. Measurement of SGOT and SGPT becomes very important because these liver enzymes are the most important liver enzymes to represent groups or transaminase aminotransferase enzyme, which catalyze the keto acids into amino acids by transfer of amino groups. Serum enzymes are the most commonly used and sensitive biochemical markers for the assessment of liver disease. \((12)\), who reported that alcohol is a toxin that is harmful to the liver and alcoholic liver disease and it is one of the leading causes of alcohol-related death. While \((13)\), found consumption of alcohol causes several pathological changes in the liver. In respect to catalase there was significant difference between case and control group \((363.42 \pm 214.61 \text{ vs. } 101.51 \pm 51.64)\) at \((P<0.01)\) respectively as show in figure 2.

![Figure 2: Comparison of catalase in serum samples of alcoholic and non-alcoholic males](image2)
The enzyme catalase has also been shown to oxidize ethanol into acetaldehyde within the peroxisomes. This process is hydrogen peroxide dependent. However, under normal physiological conditions, catalase plays only a minor role in ethanol metabolism, but its contribution might be enhanced in the presence of higher amounts of hydrogen peroxide. Furthermore, catalase may be an alternative metabolic pathway for ethanol oxidation within the brain, where ADH and CYP2E1 appear to be of minor importance for ethanol metabolism.

reported that the catalase activity increased in lower concentration of alcohol exposure, but in higher concentration of alcohol exposure catalase activity decreased compared to control groups. This finding suggested that catalase activity in the liver is changeable.

80-90% of alcohol breakdown in the liver results in the formation of acetaldehyde whose further metabolism in the cells leads to reactive oxygen species production (ROS). Acetaldehyde itself is a mutagenic and carcinogenic byproduct. It binds with DNA and interferes with DNA synthesis and repair mechanism. Furthermore, it results in tumor development.

Distributions of Genotypes and Allele Frequency of the 5-HTTLPR Polymorphisms in the SLC6A4 Gene

The distribution of the observed SLC6A4 gene genotypes and alleles frequencies in the control and cases individuals are shown in table (2). The highest genotype in the case group was homozygous LL(74.00%), while(18.00%) for homozygous SS genotype, and LS (8.00%).

<table>
<thead>
<tr>
<th>Genotype of 5-HTTLPR</th>
<th>Cases No. (%)</th>
<th>Control No. (%)</th>
<th>Sig.</th>
<th>O.R. (95% C.I.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>9 (18.00%)</td>
<td>5 (10.00%)</td>
<td>0.0438 *</td>
<td>0.662 (0.78-1.56)</td>
</tr>
<tr>
<td>LS</td>
<td>4 (8.00%)</td>
<td>37 (74.00%)</td>
<td>0.0001 **</td>
<td>1.750 (0.86-1.71)</td>
</tr>
<tr>
<td>LL</td>
<td>37 (74.00%)</td>
<td>8 (16.00%)</td>
<td>0.0001 **</td>
<td>1.697 (0.86-1.64)</td>
</tr>
<tr>
<td>Total No.</td>
<td>50</td>
<td>50</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Allele</td>
<td>Frequency</td>
<td>Frequency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>0.22</td>
<td>0.47</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>L</td>
<td>0.78</td>
<td>0.53</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

* (P<0.05), ** (P<0.01).

The results from table (2) show significant differences in the frequencies of SLC6A4 gene (LL) in the control and case groups (at P-value 0.01). The results raveled that the odd ratio for the LL genotype was 1.697 indicating that homo mutant genotype were a higher risk of alcoholism than the wild type SS. This result showed that L
allele frequency was (0.78) in alcoholism group and (0.53) in control group whereas S allele frequency was (0.22) in the alcoholism group and (0.47) in the control group as shown in table (2). So, the results showed there was a significant difference in allele frequencies of \( SLC6A4 \) gene between case and control (\( P<0.01; \) OR=1.697; 95% CI=0.86-1.64).

Alleles of the 5-HTTTLPR promoter have either a short (S) or long (L) copy of an imperfect repeat. The short or ‘S’ allele with 14 repeats was shown to have lower transcriptional activity than the long or ‘L’ allele with 16 repeats (20, 21). (22) reported that the ‘L’ allele is associated with a predisposition to lower level of response to alcohol, which is in turn associated with the onset of alcoholism. While several previous studies that suggested an association of the S allele with alcohol and drug dependence (23, 24, 25). (26) found out that there is an association between the 5-HTTLPR ‘L’ allele and the increased serotonin and platelet uptake pharmacologically; the serotonin transporter spans the plasma membrane 12 times.

### Association between genotype of 5-HTTLPR and parameters

The association between 5-HTTLPR and SGOT, SGPT and catalase were investigated. The current study shows that the presence of an SGOT was significantly associated with genotype of 5-HTTLPR (SS, SL, LL) among cases at \( (p < 0.05) \), while SGPT was significantly among control at \( (p < 0.05) \). Regarding the association between genotype of 5-HTTLPR and catalase, there were no differences among case and control groups, table (3).

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotype of 5-HTTLPR</th>
<th>SGOT Mean ± SD</th>
<th>SGPT Mean ± SD</th>
<th>Catalase Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>case</td>
<td>SS</td>
<td>29.16 ± 15.56 b</td>
<td>55.04 ± 15.05</td>
<td>389.82 ± 263.95</td>
</tr>
<tr>
<td></td>
<td>SL</td>
<td>40.23 ± 19.95 b</td>
<td>47.84 ± 21.29</td>
<td>379.37 ± 243.08</td>
</tr>
<tr>
<td></td>
<td>LL</td>
<td>61.06 ± 32.28 a</td>
<td>48.67 ± 26.12</td>
<td>273.13 ± 226.82</td>
</tr>
<tr>
<td></td>
<td>LSD value</td>
<td>20.77 *</td>
<td>21.71 NS</td>
<td>186.97 NS</td>
</tr>
<tr>
<td></td>
<td>SL</td>
<td>20.50 ± 15.58</td>
<td>9.35 ± 2.15 b</td>
<td>111.22 ± 59.37</td>
</tr>
<tr>
<td></td>
<td>LL</td>
<td>20.20 ± 11.18</td>
<td>15.80 ± 9.38 ab</td>
<td>104.24 ± 55.93</td>
</tr>
<tr>
<td></td>
<td>LSD value</td>
<td>8.67 *</td>
<td>55.92 NS</td>
<td></td>
</tr>
</tbody>
</table>

* (\( P<0.05 \)), NS: Non-Significant. Means having with the different letters in same column differed significantly

The results in the present study indicated that genetic polymorphisms of the 5-HTTLPR and SGOP in humans are linked to alcohol consumption and the incident of alcohol abuse.

Regarding the association between genotype of 5-HTTLPR and catalase, may be, duration of ethanol exposure and genetic background appear to be important variables in considering whether or not catalase changes as a response to ethanol.

Several studies have examined whether the 5-HTTLPR polymorphism (L and S variants, LL, LS and SS genotypes) interacts with environmental risk factors to predict drinking outcomes (27, 28).

A repeat length polymorphism (5-HTTLPR) in the promoter of this gene has been shown to affect the rate of serotonin uptake and may play a role in drug dependence and other chronic neurological diseases (20, 21).

Some studies have suggested a possible involvement of the 5-HTTLPR genotype with alcoholism (22), smoking (29), suicidal behavior (30), and depression (31).

### Conclusion

Researchers worldwide reported that there are several underlying genetic factors that influence the development of alcoholism among individuals. However, to date there is only few published reports on this matter in alcoholism among Iraqi individuals.
In this study there was a significant positive correlation between the SGOT activity and genotypes of 5-HTTLPR (SS, SL, LL). There was a significantly increased levels of liver enzymes (GOT and GPT) and catalase in serum samples of alcoholic compared to non-alcoholic males. The results in the present study indicated that genetic polymorphisms of the 5-HTTLPR and SGOT in humans are linked to alcohol consumption and conditions of alcohol abuse.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest:** Non

**Funding:** Self-funding

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Sensitive Simultaneous Estimation of Atorvastatin. Ca in Pure and Dosage Forms Via Developed CFIA Using 1,2 Naphthoquinone-4-Sulfonate as a Suitable Organic Agent

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Abstract

Objectives: A sensitive visible spectrophotometric method and FIA/merging zones technique was developed for the determination of atorvastatin calcium in pure material and tablet dosage form.

Method: Atorvastatin calcium has a free carboxylic moiety in its structure, which when being deprotonated in basic medium facilitates the association of the reagent with the drug. This method was based on the formation of red colored chromogen of drug with 1,2-Naphthaquinone-4-sulfonate(NQS) in basic medium (NaOH). The absorbance of the chromogens was measured at their respective wavelengths of maximum absorbance against the corresponding reagent blank.

Results: The red colored product is directly completed in basic medium and exhibits maximum absorption at 525 nm. Different factors affecting the formation of the product and optimized in order to obtain the best conditions for the experiment and its stability were studied. Method validation was done over a concentration range of 2-10 and 1-20 μg/mL for batch and FIA method respectively.

Keywords: Atorvastatin calcium; 1,2-Naphthoquinone-4-sulfonate, sodium hydroxide; Pharmaceutical formulation; CFIA/merging zones technique.

Introduction

ATRV.Ca [R-(R, R*)]-2-(4-flurophenyl)-β,δ-dihydroxy- 5(1-methylethyl)-3-phenyl-4-[phenylamino]carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) is the most commonly occurring drug in commercially available pharmaceutical formulations used for the clinical treatment of hypercholesterolemia (¹). Several methods have been described for the determination of ATRV.Ca HPTLC (²), (HPLC) in different pharmaceutical preparations, either alone (³-⁸) or with other active ingredients (⁹-¹⁷), electrochemical (¹⁸,¹⁹), spectrofluorimetric (²⁰) and capillary electrophoresis (²¹) methods have been developed for the analysis of ATRV.Ca in pharmaceutical preparations. Various spectrophotometric methods have been reported for the determination of ATRV (⁹,¹⁵,²²-²⁶) from its individual and combined formulations with other active ingredients. The official procedures in pharmaceutical preparations utilize non-aqueous titration method (²⁷). Kinetic methods have certain advantages in pharmaceutical analysis regarding selectivity and elimination of additive interferences, which affect direct spectrophotometric methods. Some specific advantages that the spectrophotometric FIA methods possess are as follows (²⁸).

- High selectivity since they involve the measurement of the absorbance as a function of reaction time instead of measuring the concrete absorbance value.

- Simple and fast methods because some experimental steps such as filtration, extraction, etc.

-Other active compounds present in the commercial dosage forms may not interfere if they are resisting the chemical reaction conditions established for the proposed method.

- Colored and/or turbid sample background may possibly not interfere with the determination process.

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Materials and reagent

A standard solution of ATRV.Ca (C_{66}H_{68}CaF_{2}N_{4}O_{10}= 1155.34 g mol^{-1}, Sigma Aldrich). A 0.05 g of pure ATOR was dissolved in 100 mL methanol to prepare 500 μg/mL of standard ATRV.Ca. A standard stock solution of NQS (C_{6}H_{4}COCOCH:CSO_{3} Na = 260.20 g mol^{-1}, Fluke) A 0.05 M of Reagent was prepared by weighing a 1.3 g of reagent and dissolving in distilled water and made up to 100 mL with it. A stock solution of NaOH (40 g mol^{-1}, BDH) A NaOH 1M was prepared by weighing a 4g of oxidant and dissolving in distilled water and made up to 100 mL with it.

Instrumentation

A Optima, Photomech 301-D^{+}, UV-Visible Spectrophotometer single beam recording spectrophotometer (Japan) was used for performed all absorbance and spectral measurements of FIA procedures, for the absorbance measurements as peak height through Kompensograph C1032, Siemens or absorbance with digital multimeter (DT9205A, China). Inside the detection unit, there is a flow cell (quartz silica (QS), 1 cm) with 80 μL internal volume. A Shimadzu UV-1800 (Japan) double-beam spectrophotometer were used for batch procedure, and quartz cuvette with an optical path length of 1 cm. A one channel manifold was employed for the FIA/merging zones system. A peristaltic pump of four channels (Shenchen, LabM1) used for pumping the distilled water as a carrier stream of through the valve (homemade, six-three injection valve (merging zone version)), which moves at 90° and three Teflon loops were loaded with the sample solutions and reagent. Mixing coil that was manufactured from glass with 2 mm (I.D). A single channel manifold system in FIA was shown in Figure.1.

Assay procedure for tablets

The solutions of pharmaceutical preparations by appropriate amount equivalent 0.02 g of the each sample was weighting that be equal to 200 μg mL^{-1} of resulting powder were dissolved in 100 ml volumetric flask with 25 mL of methanol for and then shaken and filtered into a volumetric flask of 100 mL. The residue was washed and diluted to volume with distilled water to gain 200 μg/mL of statin drugs.

Mechanism of the Reaction

The suggested mechanism of this reaction of ATRV.Ca with (NQS) in basic medium to form a red complex directly as shown in scheme (I). The stoichiometry of the reaction between ATRV.Ca and NQS was investigated (22).
Result and Discussion

Batch spectrophotometric determination: In the subsequent experiments, 4 µg mL⁻¹ of ATRV.Ca was taken in 10 mL final volume and performed by changed one factors at a time and keeping the other parameters fixed and observing the effects of the product on the absorbance.

Concentration of NQS:

The effect of various concentration of NQS was investigated using different concentration ranging from (0.001-0.01 M). A concentration of 0.005 M reagent gave the highest absorbance and was chosen for further experiments.

Concentration of sodium hydroxide: The effect of concentration of sodium hydroxide was investigated by carrying out the reaction using different volumes of NaOH ranging (0.005-0.2 M). The maximum absorbance was obtained upon 0.05 M.

Calibration curve of classical method:

The impact of using different concentration of ATRV.Ca (1, 2, 2.3, 2.5, 3, 4, 5, 6, 7, 8, 10, 12) µg mL⁻¹ were examined with stabilized the other parameters. Transfer set of volumetric (10 ml) contain 2.5 mL of (NQS) (0.02 M) followed by 1 mL of NaOH (0.5 M) then an increasing volumes from standard solutions (100 µg.ml⁻¹). The solutions had been diluted to the marked using distilled water. The reaction mixture measured the maximum absorption of the colored product at 525 nm. The standard curve was constructed and linear range (2-8) µg.mL⁻¹ for the determination of ATRV.Ca, as shown in Figure (2).
Calculations of stability constant:

\[ K = 1 - \alpha / \alpha^2 \]  

(1), \( \alpha \) (degree of dissociation) can be written as follows:

\[ \alpha = A_m - A_s / A_m \]  

(2), \( A_m \); \( A_s \) are the values of absorbance of the aqueous solution including a more than enough and stoichiometric amount of the reagent.

**Optimization of the FIA system conditions**

Initial studies were directed towards the optimization of the experimental conditions for FIA system.

**Effect of reagent and basic medium:** Optimum concentration of the reagent was studied by injecting different concentrations (0.005-0.08) M using IV. The results indicated that the 0.05 M gave the good repeatability with highest value of absorbance.

NaOH found to be a useful basic medium for this reaction, different concentrations of NaOH were also studied in the range of 0.01 to 0.08 M. The result referred to increase the value of absorbance with increasing the concentrations of basic medium up to 0.02 M and after this concentration the value of absorbance decreased. As a result, 0.02 M was chosen for the subsequent experiments.

**Effect of physical parameters**

**Effect of optimum total flow rate**

Optimum flow rate was studied using a range changed flow rates (1.2-2.6) mL min\(^{-1}\). The result demonstrates that a flow rate of 2.16 mL min\(^{-1}\) gave the highest absorbance value.

**Effect reaction coil length and injection volume**

Optimum length of reaction coil was studied in range of 85-250 cm. A best absorbance with acceptable repeatability was gained from the length of 85 cm. Absorbance decreased upon using a coil length of more than 85 cm.

Various volumes of injector loop were tested in this study. Effect of injected sample volume (L\(_1\)) was changed (58.875, 68.687, 88.312 and 127.562) µL and the volume of injection reagent (L\(_2\)) also studies was in deferent volume (68.687-127.562) µL. a 58.875, 68.687
µL for L₁, L₂ respectively was used in the next experiments.

Method validation

The linearity of the calibration graph for FIA method was obtained by injecting a series of solutions of ATRV. Ca (1-20 µg mL⁻¹) prepared from stock solution (100 µg mL⁻¹) with 0.02 M of basic medium as shown in figure (3). A portion of NQS (0.005 M) was injected as summarized in Table 1. These small points were referred to high reproducibility and repeatability of the developed FIA contrasted with the batch procedure.

![Figure 3. Linear calibration curve for determination of atorvastatin calcium with NQS using the developed FIA system.](image)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Batch method</th>
<th>FIA method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear range (µg mL⁻¹)</td>
<td>2-8</td>
<td>1-20</td>
</tr>
<tr>
<td>Regression equation</td>
<td>y = 0.1532x + 0.2134</td>
<td>y = 31.929x + 11.461</td>
</tr>
<tr>
<td>Correlation coefficient (r)/ r²</td>
<td>0.9945</td>
<td>0.9983</td>
</tr>
<tr>
<td>Linearity (r² %)</td>
<td>98.91</td>
<td>99.67</td>
</tr>
<tr>
<td>Relative standard deviation (RSD %)</td>
<td>0.21 (at 5 ppm)</td>
<td>0.3 (at 10 ppm)</td>
</tr>
<tr>
<td>Slope (b); (mL·µg⁻¹)</td>
<td>0.1532</td>
<td>31.929</td>
</tr>
<tr>
<td>Intercept (a); (a = y– b x)</td>
<td>0.2134</td>
<td>11.461</td>
</tr>
<tr>
<td>Standard deviation of intercept (Sa)</td>
<td>7.07 × 10⁻⁵</td>
<td>4.47 × 10⁻⁴</td>
</tr>
<tr>
<td>Confidence limit of intercept (a) = a ± tSa</td>
<td>0.2134 ± 0.0007</td>
<td>11.461 ± 0.163</td>
</tr>
<tr>
<td>Standard deviation of slope (Sb)</td>
<td>8.49 × 10⁻⁴</td>
<td>5.74 × 10⁻⁴</td>
</tr>
<tr>
<td>Confidence limit of slope (b) = b ± tSb</td>
<td>0.1532 ± 0.0002</td>
<td>31.929 ± 0.0049</td>
</tr>
<tr>
<td>Standard deviation of the residuals;</td>
<td>0.38 × 10⁻⁴</td>
<td>0.015</td>
</tr>
<tr>
<td>Average of recovery (%)</td>
<td>99.66</td>
<td>100.4</td>
</tr>
<tr>
<td>Limit of detection (LOD)</td>
<td>0.06</td>
<td>0.002</td>
</tr>
<tr>
<td>Limit of quantification (LOQ)</td>
<td>0.2</td>
<td>0.006</td>
</tr>
<tr>
<td>Sample through put (h⁻¹)</td>
<td>10</td>
<td>68</td>
</tr>
</tbody>
</table>
Application of the proposed method using pharmaceutical:

The proposed batch and FIA method was successfully applied for estimation ATRV.Ca in tablets by the analysis of three types in two different concentrations of ATRV.Ca tablets and the results are listed in Table 2. In the direction of assessing the proficiency of the method. The statistical comparison between proposed and official methods using the student t- and F-test \(^{(27)}\) indicated that the calculated values for F-test were (2.57) and (1.22), t-test values were (2.08) and (1.13) for the FIA and batch methods, respectively, were less than the theoretical one of F-test = 6.388 \((n1 + n2 – 2 = 6)\) and t-test = 2.31.

<table>
<thead>
<tr>
<th>Dosage form</th>
<th>Proposed methods</th>
<th>FIA-merging zones</th>
<th>Official method recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Batch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present conc.</td>
<td>Rec (%)</td>
<td>RSD (%)</td>
<td>Present conc.</td>
</tr>
<tr>
<td>(µg mL(^{-1}))</td>
<td></td>
<td></td>
<td>(µg mL(^{-1}))</td>
</tr>
<tr>
<td>AVAS Tablets (10 mg/tablet) 3</td>
<td>100.30</td>
<td>0.41</td>
<td>10</td>
</tr>
<tr>
<td>MICRO LABS LIMITED</td>
<td>5</td>
<td>99.92</td>
<td>15</td>
</tr>
<tr>
<td>AVAS Tablets (20 mg /tablet) 3</td>
<td>101.30 0.20</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>MICRO LABS LIMITED</td>
<td>5</td>
<td>101.00 0.19</td>
<td>15</td>
</tr>
<tr>
<td>LIPODAR Tablets (10mg /tablet) 3</td>
<td>99.00 0.21 Dar Al Dawa, Na,ur - Jordan</td>
<td>10</td>
<td>98.20</td>
</tr>
<tr>
<td>5 99.40 0.18</td>
<td></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>LIPODAR Tablets (20mg /tablet) 3</td>
<td>99.67 0.52 Dar Al Dawa, Na,ur – Jordan</td>
<td>10</td>
<td>101.00</td>
</tr>
<tr>
<td>5 101.20 0.089</td>
<td></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>ATEROZ Tablets (20mg /tablet) 3</td>
<td>98.67 0.93</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>bilim 5 100.60 0.04</td>
<td></td>
<td></td>
<td>15</td>
</tr>
</tbody>
</table>

**Conclusion**

The developed methods were selective, rapid, simple and inexpensive and exhibits a fair degree of accuracy and precision. The method does not involve any critical reaction conditions. The proposed method can serve as an alternative method for the routine analysis of ATRV.Ca in pure drug and in pharmaceutical formulations. The methods is based on formation of a red condensation adduct upon reaction of ATRV.Ca and NQS in (NaOH). The method has low detection limit and high sample throughput. The proposed methods that followed Beer’s law and give a good application for the pharmaceutical preparation. The wide applicability of the FIA method for daily quality control is well proven by analyzing the assay of ATRV.Ca at effect concentration level in dosage forms.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq
Conflict of Interest: Non

Funding: Self-funding

References


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48: 120–126.


Gall Bladder Wall Thickness: Sonographic Accuracy and Laparoscopic Cholecystectomy Conversion Rate, Evaluated by Histopathology

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Abstract

Background: Preoperative prediction of a difficult laparoscopic cholecystectomy can help the patient as well as the surgeon to prepare better for intraoperative risk and the risk of conversion to open Cholecystectomy.

Aim of study: Evaluation the impact of gall bladder wall thickness, on the outcome of laparoscopic cholecystectomy and conversion rate to open cholecystectomy assessed by sonography preoperative and postoperative measurement of gall bladder wall thickness by histopathology.

Patients and Methods: A prospective study conducted in the surgical unit, Department of surgery, Baghdad Teaching Hospital between November 2010 and November 2011. Abdominal Sonography performed in 110 consecutive patients before laparoscopic cholecystectomy. The surgeon re-verified sonographic finding in operative room, and postoperatively, the gall bladder specimens were sent for histopathological measurement of wall thickness.

Results: Out of 110 patients with cholecystolithiasis on sonography, we encountered easy laparoscopic cholecystectomy in 80 patients (72.7%), difficult laparoscopic cholecystectomy in 24 (21.8%) and the procedure was converted to open cholecystectomy in six patients (5.5%). The difference between Sonographic and histological measurement was within 1 mm in 102 patients (92.7%), and the other 8 patients was with 1.5 mm (7.3%) with sensitivity of (100%), specificity of (83.3%) and accuracy of (97%).

Conclusion: An accurate preoperative diagnostic sonography is mandatory for planned laparoscopic gall bladder surgery to provide information for the selection of the most appropriate approach and avoid intraoperative difficulties and surprises. On sonography gall bladder wall thickening is the most sensitive indicator of technical difficulties during laparoscopic cholecystectomy. Such difficulties may require conversion to laparotomy.

Keywords: Laparoscopic cholecystectomy, gall bladder wall thickness, sonography, Iraq.

Introduction

Cholelithiasis has a high prevalence. Although cholelithiasis only becomes symptomatic in about 50% of patients, cholecystectomy is a common surgical procedure (1). Gallstones are one of the major causes of morbidity in Western society. Prevalence of people with gallstones, whether symptomatic or asymptomatic, varies from 5 to 22% (2). In Iraq, operations of gallbladder (GB) represent a considerable fraction of total operations conducted in hospitals. This indicates that the disease is relatively important in Iraq (3). Recently, laparoscopic cholecystectomy (LC) has become the gold standard for treatment of symptomatic gallstones, due to lower morbidity, shorter hospital stay, earlier return to regular daily activities, less postoperative pain and a significant reduction in the incidence of wound complications and postoperative ileus has been documented in patients undergoing LC (4, 5). In addition to numerous advantages, also technical limitations of laparoscopy should be mentioned, which - in the presence of chronic inflammation resulting in
pericystic adhesions and conglutination – increase the risk of undesirable conversion from LC to open surgery (6). The severity of acute inflammatory change influences the degree of surgical difficulty. GB wall thickening and pericholecystic fluid are indicators of inflammation in patients with acute cholecystitis (7). The most common risk factors for conversion include a thickened GB wall, past acute cholecystitis, diabetes mellitus, past upper gastrointestinal tract surgeries, age > 65 years and male gender (8, 9). The selection of the patient who will undergo LC is important, and the most frequently used method other than the clinical evaluation, is radiological examination (ultrasonography) (2). Preoperative classification of patients into a high risk group would be an objective factor facilitating the surgeon’s decision on possible conversion (9). A preoperative GB ultrasound, which documents a thick GB wall (> or = 3 mm) with calculi, is a clinical warning for a difficult LC which may require conversion to an open surgery. In a study, it was found that the rate of conversion was 60% in case of thickened GB wall while 12% in case of normal GB wall (10). Variable results have been reported in the past about the sensitivity, specificity, positive predictive value, and accuracy of GB wall thickening as an indicator of surgical conversion (7).

The success of any laparoscopic operation depends on both proper patient selection, and the technical skill and experience of the laparoscopist (11). The aim of the study was to evaluate the impact of GB wall thickness on the outcome of LC and the conversion rate to open cholecystectomy assessed by preoperative sonography and postoperative measurement of GB wall thickness by histopathology.

Patients and Methods

Study Design and Setting: This was a prospective study that was conducted in the surgical unit, Department of Surgery, Baghdad Teaching Hospital during a period of one year from Nov, 2010 – Nov, 2011.

Study Population and sample size: The study included patients with feature of chronic calculus cholecystitis who were prepared for LC, so the total number was 110. Patients who had previous abdominal surgery and features of acute cholecystitis (clinically and by investigation) were excluded.

Workup: All patients were evaluated by sonography after fasting at least six hours, the wall of GB was carefully evaluated and consider as thick when it is (> 3mm), size and capacity of GB, pericystic fluid collection and biliary system status was evaluated as well as number of GB stones also recorded. Hematological and biochemistry investigation were done. GB wall thickness was measured postoperatively by histopathology; grossly and microscopically as. Initial Procedure of histopathological examination done by.

Measurements:

- GB: length × maximum diameter (cm).
- Cystic duct: length × maximum diameter (cm).
- Lymph node: number and maximum diameter (cm).
- Open longitudinally from the fundus towards the cystic duct with blunt-ended scissors, draining off the bile and noting any contents.
- Photograph if appropriate.
- Paint the external serosal and adventitial aspects if there is any suspicion of tumor.
- Fixation by immersion in 10% formalin for 36 – 48 hours.

The difficulty of procedure was evaluated by:

1. Clarity of calot’s triangle (peritoneal adhesion) length and width of cystic duct.
2. Handling of GB during procedure and ability to perforate it.
3. Dissection of GB from its liver bed and bleeding from it.
4. Extraction of GB to outside.

All patients were undergoing surgery which was done by senior general surgery and resident using closed methods with four ports. Histopathological examination done by senior histopathology. LC considered easy when there is minimal adhesion involving the omentum, only attaches to the fundus and body of GB, and easily separated. Difficult LC when there is sever adhesion involving calot’s triangle.

Statistical analysis: The data analyzed using Statistical Package for Social Sciences (SPSS) version 25. The data presented as mean, standard deviation
and ranges. Categorical data presented by frequencies and percentages. Chi–square test was used to assess statistical association between certain variables and GB Wall thickness. A level of p–value less than 0.05 was considered significant.

Results

In this study, mean age of patients was 42.7 ± 8.4 years; and 82.6% were females. By U/S, 64.5% of patients had GB with wall thickness ≤ 3 mm. We noticed that LC was easy in 72.7% of cases, difficult in 21.8%, and converted to open surgery in 5.5% of cases as shown in table (1).

Table 1: Distribution of study patients by certain characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. (n=110)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 30</td>
<td>24</td>
<td>21.8</td>
</tr>
<tr>
<td>30 - 49</td>
<td>64</td>
<td>58.3</td>
</tr>
<tr>
<td>≥ 50</td>
<td>22</td>
<td>19.9</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>19</td>
<td>17.4</td>
</tr>
<tr>
<td>Female</td>
<td>91</td>
<td>82.6</td>
</tr>
<tr>
<td>GB Wall thickness (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 3</td>
<td>71</td>
<td>64.5</td>
</tr>
<tr>
<td>&gt; 3</td>
<td>39</td>
<td>35.5</td>
</tr>
<tr>
<td>Type of operation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Easy LC</td>
<td>80</td>
<td>72.7</td>
</tr>
<tr>
<td>Difficult LC</td>
<td>24</td>
<td>21.8</td>
</tr>
<tr>
<td>Converted to open surgery</td>
<td>6</td>
<td>5.5</td>
</tr>
</tbody>
</table>

The difference between sonographic and histopathologic measurement was below 0.5 mm in 80 patients (72.5%) and it was between 0.5 and 0.99 mm in 22 patients (20%), so it was within 0 – 1 mm in 102 patients (92.7%), and in other 8 patients the difference was within 1.5 mm from GB wall thickness as shown in table (2).

Table 2: Difference of histopathological and ultrasound measurement of gall bladder wall thickness.

<table>
<thead>
<tr>
<th>Difference between measurements (mm)</th>
<th>No. (n=110)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No difference</td>
<td>44</td>
<td>40.0</td>
</tr>
<tr>
<td>0.1 – 0.49</td>
<td>36</td>
<td>32.5</td>
</tr>
<tr>
<td>0.5 – 0.99</td>
<td>22</td>
<td>20.0</td>
</tr>
<tr>
<td>1 – 1.5</td>
<td>8</td>
<td>7.5</td>
</tr>
</tbody>
</table>

In table 3, 83.3% of cases who were converted to open surgery had GB wall thickness > 3 mm by U/S with a significant association (P= 0.001) between GB Wall thickness by U/S and type of operation.

Table 3: Association between GB Wall thickness by U/S and type of operation

<table>
<thead>
<tr>
<th>Type of operation</th>
<th>GB Wall thickness by U/S (mm)</th>
<th>Total (%) n= 110</th>
<th>P - Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤ 3 (%) n= 71</td>
<td>&gt; 3 (%) n= 39</td>
<td></td>
</tr>
<tr>
<td>Easy LC</td>
<td>64 (80.0)</td>
<td>16 (20.0)</td>
<td>80 (72.7)</td>
</tr>
<tr>
<td>Difficult LC</td>
<td>6 (25.0)</td>
<td>18 (75.0)</td>
<td>24 (21.8)</td>
</tr>
<tr>
<td>Converted to open</td>
<td>1 (16.7)</td>
<td>5 (83.3)</td>
<td>6 (5.5)</td>
</tr>
</tbody>
</table>

In table 4, 90% of cases who complained from GB perforation had GB wall thickness > 3 mm by U/S with a significant association (P= 0.001) between GB Wall thickness by U/S and postoperative complication.
Table 4: Association between GB Wall thickness by U/S and postoperative complication

<table>
<thead>
<tr>
<th>Postoperative complication</th>
<th>GB Wall thickness by U/S (mm)</th>
<th>Total (%)</th>
<th>P - Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤ 3 (%) n= 71</td>
<td>&gt; 3 (%) n= 39</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>68 (72.3)</td>
<td>26 (27.7)</td>
<td>94 (85.5)</td>
</tr>
<tr>
<td>GB Perforation</td>
<td>1 (10.0)</td>
<td>9 (90.0)</td>
<td>10 (9.1)</td>
</tr>
<tr>
<td>Bleeding</td>
<td>2 (33.3)</td>
<td>4 (66.7)</td>
<td>6 (5.5)</td>
</tr>
</tbody>
</table>

Discussion

Since the Introduction in 1985, LC had been the procedure of choice in treatment of symptomatic gall stone (12). But some of the planned LC needs conversion due to various factors, it would be useful in advance to know which one would require conversion, so that experienced laparoscopic surgeon could be scheduled to minimize conversion rate. And since the 1970s, ultrasound has become known as a quick, non-invasive and reliable tool to diagnose GB disease (13, 14). Ultrasound is very sensitive for the diagnosis of gall stones, but few data are available to assess its diagnostic value for the GB wall thickness (15). We assessed the value of sonography for patients with gall stone disease prior to LC. This study corroborates the well-established high accuracy (97%) of sonography for assessing the thickness of GB wall thickness.

In this study, we found that increase GB wall thickness on preoperative ultrasound which encountered in 39 patients out of 110 patients (35.5%) were associated with increase operative difficulty in 18 patients out of 39 patients, and our conversion rate to open surgery in six patients out of 110 patients (5.5%) was within the range reported by several other studies (1 – 10%) as in Indian one conducted in 2017 with a report of conversion rate of 10% (16), in USA in 2010 were the rate was 9% (17), and a local study in Iraq in 2007 (18) where the rate was 5%. In this study, GB wall thickness significantly determines the difficulty during surgery. We found that increase GB wall thickness (> 3 mm) on preoperative ultrasound which encountered in 39 patients (35.5%) in comparison to those with thin GB wall thickness (≤ 3 mm) 71 patients (64.5%) was associated with increase operative difficulty and this result was in consistent with a result found by Adwan MK et al study in 2015 (3) and with a study conducted by Sharma N et al in 2015 (19) when reported that gall bladder wall thickening can predict difficulty during cholecystectomy, we found that thickened gall bladder wall are the most accurate predictors of potential operative difficulty. GB wall thickening was a sensitive indicator of technical difficulties and the risk of conversion to open cholecystectomy. GB wall thickness is related to the inflammation and fibrosis that follow previous attach of cholecystitis and thus may reflect difficulty in delineation of the anatomy during surgery (20). In conclusion, LC can be accomplished successfully with low morbidity in most patients with cholecystitis, those patients with increased GB wall thickness on preoperative ultrasonography are at high risk for conversion to open surgery. An accurate preoperative diagnostic tool is mandatory for planned laparoscopic GB surgery to provide information for the selection of the most appropriate approach and to avoid intraoperative difficulties and surprises.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

References

Evaluating the Effect of Addition of Titanium Dioxide Nanoparticle on Some Physical Properties of Flowable Composite Resin

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¹Hawler Medical University, College of Dentistry, conservative dentistry department/Iraq

Abstract

Objective: The flowable dental composite resins were introduced to the dental specialty because of the advantages they possess over the conventional composite resins. The use of nanotechnology in the dentistry field is one of the growing innovations in recent years. The aim of the present study was to evaluate certain physical properties of flowable dental composite after incorporation of titanium dioxide nanoparticles (TiO2NPs).

Materials and Method: In the present study, TiO2NPs at 1.25 % and 2.5% concentrations were added to flowable composite, while the unmodified composite was used as control. Then the physical properties of the control and modified composite resins, including flowability, radiopacity and water sorption and solubility were tested. Data were analyzed with One way ANOVA, using SPSS 20.

Results: The results showed that there was statistically significant difference among the tested groups regarding flowability and radiopacity (P<0.05). In addition, there was no significant difference among control group and TiO2 modified groups regarding water sorption and solubility.

Conclusion: Based on the results of the present study, a flowable dental composite was successfully reinforced with TiO2. Incorporation of small weight percentages of this nanofiller exhibited properties similar to the control material regarding water sorption and solubility. The flowability was slightly reduced and radiopacity of the reinforced composites was increased, these changes were acceptable for clinical applications and below ISO standards limits.

Keywords: Flowable Composites, Titanium Dioxide nanoparticles, flowability, radiopacity, water sorption and solubility.

Introduction

Currently composite resins are materials used in restorative dentistry, since there are many revolution and improvements such as use of different novel particles, flowable composite resin because of their advantages over the conventional composite resins were introduced to the dental specialty. These advantages includes, simple application technique, easy handling properties, increased flowability, better adaptation to the internal cavity wall and higher elasticity, in addition, the use of flowable composites resins reduces the amount of the cavity preparation and is recommended for minimal invasive dentistry (¹).

One of the most important innovations in the dentistry field in recent years is the use of nanotechnology. The mechanical, physical and optical properties of conventional composite resins have been improved by addition of inorganic nanoparticles such as silver, zinc, titanium dioxide and silica (²).

Flowability is used to describe how quickly materials flow in a certain period of time, whereas viscosity is the material’s resistance to flow. Dental composites are viscoelastic materials. They share criteria of both viscous materials (e.g., oils) and elastic materials (e.g., metals).
Radiopacity of dental restorative materials is an important indicator for accurate diagnosis and treatment planning. It is essential to use materials with adequate radiopacity in order to distinguish them from the natural tooth structures. Moreover, dental restorations should be radiopaque enough to detect overhanging margins, recurrent caries, restoration contour, proximal contacts (3).

The sorption and solubility properties are critical properties regarding biocompatibility concerns of releasing monomer and in relation to the stability of the composites due to degradation from the uptake of solvents and the wash-out of ingredients of materials (4).

Most of the studies involving addition of nanoparticles to dental composites resins have mainly focused on their anti-bacterial effects and the information regarding their physico-mechanical properties are limited. TiO2 are fine, non-toxic, chemically stable, and exhibits a high photocatalytic effect in addition to their high biocompatibility and pleasant color. TiO2NPs have large surface area that facilitates load transfer from resin matrix to nanoparticles thereby resulting in better mechanical properties of the reinforced composites (5).

Mohammed et al (2019) studied the effect of TiO2NPs on physico-mechanical properties of flowable dental composite resins by adding TiO2NPs at 1%, 2% and 3% to Tetric N Flow composite, while the unmodified composite was used as control. The developed composite was studied for functional and structural properties using FTIR, which indicated no change in the functional and structural characteristics (6).

The aim of the present study was to evaluate the effect of TiO2NPs, on some physical properties of flowable composite resins.

Method

In this study, the flowability, radiopacity and water sorption and solubility of a conventional flowable composite resin and composite resins reinforced with TiO2NPs were evaluated. For each test a total of 18 samples were evaluated. Commercially available flowable microhybrid composite resin (as control group) was used shade (A2). This material is based on dimethacrylate paste (Bis-GMA and Triethylene glycol dimethacrylate TEGDMA), without inorganic fillers. mixed with TiO2NPs 98% Purity and particle size of 50 nm at concentrations of 1.25% and 2.5 %.

Mixing was done by using a dental Micro motor, with a lentulo spiral-paste carrier #4 attached. The lentulo spiral was immersed in composite resin material – TiO2NPs, poured into a 2 ml pre-darkened syringe tube.

Such mixing was performed directly before preparation of samples for each test. Electronic balance was used to weight the percentage of nanoparticles, after one hour mixing; the mixture was injected into metal molds Curing was done by exposure to LED at 1,650 mW/cm2 for 40 seconds with a light guide held perpendicularly and within 2 mm of the material surface. Control samples were prepared and compared with two sets of samples with TiO2NPs at 1.25% and 2.5%.

The flowability testing method used in this study was according to ADA guidelines for evaluation of endodontic sealing materials (7). To evaluate the flowability of the reinforced composites, a simple test using the Gillmore needle apparatus was used. The quantity of 0.1 mL of control and each reinforced composite was dispensed between two thin glass coverslips 50 x 50 mm and 1mm thick. Flowability was evaluated by comparing the composite disc diameters after they have been sandwiched between two glass coverslips, subjected to constant weight (454 g) for 30 s and light cured for 40 s.

For radiopacity test 6 discs 15±1mm in diameter and 1± 0.1mm thick were prepared for each group. The specimens and the aluminum stepwedge were placed in the center of the film and irradiated with X-rays at (65±5) kV at a target film distance of 400 mm for 0.4 seconds at 10 mA. After developing and fixing the film, the density of the image of the specimen was compared with that of the aluminum standard using the densitometer. For Water sorption and solubility test 6 discs 15±1mm in diameter and 0.5± 0.1mm thick samples were prepared for each group using a metal molds and tested according to ADA specification (8).

Statistical Analysis

The statistical analysis was performed on SPSS program version 20.0, the descriptive statistic was done for tested groups, for verification the difference among groups analysis of variance (One-way ANOVA) with Tukey post-test.
Results

Flowability test

Table 1 presents the means and standard deviations of flowability values in conventional composite resin and composite resins reinforced with TiO2NPs at different percentages.

Based on Table 1, the Flowability of control group and 1.25% and 2.5% groups were 2.6933 cm, 2.6083 cm and 2.5067 cm respectively. The results of Table 1 revealed significant differences among the tested groups. But the difference is acceptable within the ISO standard.

Table 1: Mean values (mm), standard deviations, standard errors and 95% confidence intervals for Flowability data.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>95% Confidence Interval for Mean</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
<td>Upper Bound</td>
<td></td>
</tr>
<tr>
<td>(C)</td>
<td>6</td>
<td>2.6933a</td>
<td>0.00816</td>
<td>0.00333</td>
<td>2.6848</td>
<td>2.7019</td>
<td>2.68</td>
</tr>
<tr>
<td>1.25%</td>
<td>6</td>
<td>2.6083b</td>
<td>0.00983</td>
<td>0.00401</td>
<td>2.5980</td>
<td>2.6187</td>
<td>2.60</td>
</tr>
<tr>
<td>2.5%</td>
<td>6</td>
<td>2.5067c</td>
<td>0.01211</td>
<td>0.00494</td>
<td>2.4940</td>
<td>2.5194</td>
<td>2.50</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>2.6028</td>
<td>0.07910</td>
<td>0.01864</td>
<td>2.5634</td>
<td>2.6421</td>
<td>2.50</td>
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</tbody>
</table>

Note: Means with different letter indicates statistically significant difference (P<0.05).

Radiopacity test

Radiopacity mean values of the tested materials are presented in Table (2). As recommended by ISO (9), 2.5% group showed the highest radiopacity values 2.4760 E.q. Al thickness/mm mean value followed by 1.25% group of 2.3900 E.q. Al thickness/mm mean value of radiopacity, and 2.2833 E.q. Al thickness/mm mean value for Control group with statistical significant difference among the tested groups.

Table 2: Mean values (mm), standard deviations, standard errors and 95% confidence intervals for Radiopacity.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>95% Confidence Interval for Mean</th>
<th>Minimum</th>
<th>Maximum</th>
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<tbody>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
<td>Upper Bound</td>
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<tr>
<td>control</td>
<td>6</td>
<td>2.2833a</td>
<td>0.00816</td>
<td>0.00333</td>
<td>2.2748</td>
<td>2.2919</td>
<td>2.27</td>
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<tr>
<td>1.25%</td>
<td>6</td>
<td>2.3900b</td>
<td>0.00894</td>
<td>0.00365</td>
<td>2.3806</td>
<td>2.3994</td>
<td>2.38</td>
</tr>
<tr>
<td>2.5%</td>
<td>6</td>
<td>2.4760c</td>
<td>0.01131</td>
<td>0.00462</td>
<td>2.4641</td>
<td>2.4879</td>
<td>2.46</td>
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<tr>
<td>Total</td>
<td>18</td>
<td>2.3831</td>
<td>0.08159</td>
<td>0.01923</td>
<td>2.3425</td>
<td>2.4237</td>
<td>2.27</td>
</tr>
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</table>

Note: Means with different letter indicates statistically significant difference (P<0.05).
**Water Sorption test**

Water sorption mean values are presented in Table (3) revealed no significant differences among the tested groups. Control group showed the highest Water sorption values 1.456283 µg/mm³ mean value, followed by 1.25% group with -1.029533 µg/mm³ mean value Water sorption, and .802833 µg/mm³ mean value of 2.5% group but the difference was not significant statistically.

**Table 3: Mean values (mm), standard deviations, standard errors and 95% confidence intervals for Water sorption data test.**

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>95% Confidence Interval for Mean</th>
<th>Minimum</th>
<th>Maximum</th>
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</thead>
<tbody>
<tr>
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<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>Upper Bound</td>
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<td></td>
</tr>
<tr>
<td>control</td>
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<td>-1.456283</td>
<td>1.2018703</td>
<td>.4906615</td>
<td>-2.717569</td>
<td>-2.8037</td>
<td>.8131</td>
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<td>1.25%</td>
<td>6</td>
<td>-1.029533</td>
<td>1.3579421</td>
<td>.5543775</td>
<td>-2.454606</td>
<td>-1.8691</td>
<td>1.6260</td>
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<td>6</td>
<td>-.802833</td>
<td>1.6504075</td>
<td>.6737760</td>
<td>-2.534830</td>
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<tr>
<td>Total</td>
<td>18</td>
<td>-1.096217</td>
<td>1.3586916</td>
<td>.3202467</td>
<td>-1.771878</td>
<td>-2.8037</td>
<td>2.5000</td>
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</tbody>
</table>

Note: Means with same letter indicates statistically no significant difference.

**Water Solubility**

Solubility mean values are presented in Table (4). Test results revealed no significant differences among the tested groups. Control group showed the highest solubility values 2.516583 µg/mm³ mean value, followed by 1.25% group with 2.048467µg/mm³ mean value solubility, and 1.386167µg/mm³ mean value of 2.5% group with no statistical significant difference among the tested groups.

**Table 4: Mean values (mm), standard deviations, standard errors and 95% confidence intervals for Water solubility data test.**

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>95% Confidence Interval for Mean</th>
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<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td>Lower Bound</td>
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</tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>Upper Bound</td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>6</td>
<td>2.516583</td>
<td>.9013909</td>
<td>.3679913</td>
<td>1.570632</td>
<td>.9345</td>
<td>3.2601</td>
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<td>1.25%</td>
<td>6</td>
<td>2.048467</td>
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<td>.4726176</td>
<td>.833564</td>
<td>.8130</td>
<td>3.8530</td>
</tr>
<tr>
<td>2.5%</td>
<td>6</td>
<td>1.386167</td>
<td>1.1753861</td>
<td>.4798494</td>
<td>.152675</td>
<td>.0000</td>
<td>3.4390</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>1.983739</td>
<td>1.1257010</td>
<td>.2653303</td>
<td>1.423941</td>
<td>.0000</td>
<td>3.8530</td>
</tr>
</tbody>
</table>

Note: Means with same letter indicates statistically no significant difference.
**Discussion**

The flowability of floweble composite resin decreased slightly with addition of TiO2NPs at 1.25% and 2.5% and the results are within the ISO standards, these results are in agreement with previous study reported that it is possible to increase the nanofiller content due to their small particle sizes. This will result in minimum reduction in flowability as well as improvement in the physical properties of the nano-composites (10).

In the present study the most commonly used monomer) BisGMA and TEGDMA were used. The advantages of using BisGMA over other monomers are less shrinkage, higher modulus and reduce toxicity due to its lower volatility and diffusivity into tissue. Although, BisGMA possesses high strength and hardness, the drawback of this monomer is its high viscosity (low flow), because of hydrogen bonding interaction that occur between hydroxyl groups, which limits the incorporation of inorganic fillers and hence a low degree of conversion, Thus BisGMA diluted with other low-viscosity (high flow) monomer such as trimethylene-glycol-dimethacrylate (TEGDMA) (11) to enhance flowability of the used composite that allow incorporation of TiO2NPs that resulted in a little reduction of flowability of the tested flowable composite resin and the changes are within accepted range of ISO standards.

The radiopacity of a restorative material is an important parameter for accurate diagnosis and treatment planning. Success or failure of the restorative material is highly dependent on radiographs. Radiopacity of composite restorations has an important role in detecting recurrent caries and distinguishing the restorations from the tooth structures (12).

The 2.5% composite showed a higher value of radiopacity. Followed by 1.25% group Al, and control group with statistical significant difference among the tested groups and met the ISO standard for dental materials radiopacity. All of them had radiopacity greater than enamel (1.77 – 2 mm Al). These results agree with previous studies that recommended that the composite radiopacity should be equal to or greater than that of the enamel (13).

The atomic number of the elements is the most important factor affecting the radiopacity of dental materials (14). Radiopacity of dental composites can be increased by incorporating a higher percentage of fillers with high atomic numbers (15), TiO2NPs atomic number is 22 and density of 4.506 g/cm³ which is considered as a high atomic number and this explains the results of this study in which radiopacity of tested material increased with increasing percentage of TiO2NPs from 0% control group to 1.25% and 2.5 %..

The sorption and solubility properties are important regarding biocompatibility concerns of releasing monomer and in relation to the stability of the composites by avoiding degradation from the uptake of solvents and the wash-out of ingredients of materials (16). According to ISO standard (17), the maximum acceptable values of sorption and solubility for polymer-based restorative materials are 40 µg/mm³ and 7.5 µg/mm³ respectively. Sorption and solubility values for all samples were below the ISO standards limits so all investigated materials met the requirements of the ISO standard. The decreasing sorption and solubility with TiO2 content was not statistically significant. These results are in agreement with Robert et al (18) study who studied the effect of nanofillers on water sorption and solubility and concluded that the addition of nanofillers at low concentrations not change water sorption and solubility significantly since at lower concentrations there is no agglomeration of 50 nm TiO2NPs.

The improvement of both water sorption and solubility after addition of TiO2NPs might be attributed to numerous explanations such as nanofillers are insoluble in water so that the addition of TiO2NPs to the microhybrid flowable composite resin declines the solubility of composite resin (19).

Furthermore, titanium coupling agent incorporated in salinized TiO2NPs expands the adhesion between both resin matrix and filler particles which enhances composite resin properties and declines its water sorption and solubility (20).

Moreover, the reaction between resin (polar nature) and nanofillers certainly induce replacing the hydrophilic resin and minimizing the water uptake by decreasing this polarity through utilizing most active sites in the molecules of monomers, so the diffusivity of water particles through this material is greatly declined (21).

**Conclusion**

Based on the results, it appears incorporation of low concentration of nanofillers into conventional composite resins did not result in any changes in their water sorption...
and solubility; however, flowability and radiopacity of flowable were changed but the results are acceptable within ISO standards. Therefore, it is suggested to add small amount of nano-fillers to composite resins to prevent problems such as discoloration of composite resin or other possible changes in other physical and chemical properties.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest:** Non

**Funding:** Self-funding

**References**


Antimicrobial Activities of Green Biosynthesized Iron Oxide Nanoparticles Using F. Carica Fruit Extract

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Abstract

In the current study, iron oxide nanoparticles (IO NPs) were synthesized via modified green synthesis technique using F. carica fig extract as reducing agent. Furthermore, the microstructural properties of the synthesized IO NPs have been thoroughly elucidated. In details, the acquired NPs diameter was found to be in the range of 11-29 nm and of root mean square (RMS) of 0.64 nm using TEM and AFM techniques, respectively. Consequently, the antifungal and antibacterial activities of the synthesized IO NPs were screened against Candida and Aspergillus species as well as Gram-positive Staphylococcus aureus and Gram-negative Acinetobacter species, respectively. The presented IO NPs play an active role in the antimicrobial activities evidencing the well-organized materials system for biomedical applications.

Keywords: Iron oxide NPs, antifungal, antibacterial, F. carica extract

Introduction

Metal oxide NPs are of great importance due to a number of unique properties such as high surface-to-volume ratio, easy separation features, and low toxicity (1). IO NPs have attracted a great significance in the field of nanoscience and nanotechnology (2, 3). In conjunction with IO NPs, several applications have been proposed in the area of optoelectronics, catalysis, self-powered smart window, lithium ion batteries and diagnostic biological probes (4-8). IO NPs are extensively applied in the biomedicine field due to their low toxicity and biocompatibility. Due to the electrostatic feature of the IO NPs, they are easily interact with fungal, and bacterial living-cell membranes (9). This property allows IO NPs to harms the cell membranes of fungi or bacteria as well as inducing the toxic oxidative stress via free radical formation (10). Antimicrobial activities of IO NPs are, therefore, dependent on three essential features namely concentration of the culture media and most importantly stability (9, 10).

In this attempt, variety of approaches have been anticipated for the synthesis of IO NPs. Among these are the well-known physical and chemical methods in which preferred NPs properties can be acquired. Particularly, these methods are electrodeposition (11), conventional heating (12), hydrothermal (13, 14), wet oxidation (15), laser ablation (16), co-precipitation (17, 18), and anodization (19). However, these techniques are presented with several draw-backs such as the use of non-biodegradable stabilizing agents and toxic chemicals, and hypothetically harmful to the well-being organisms and the surrounding environment. The green synthesis technique, which is utilized in the current study, has revealed considerable advances such as environmentally friendly, unpresented toxic chemicals, and low energy and temperature conditions (20). Green synthesis technique evolves the use of naturally existing resources such as plants extracts as reducing fuel (21). Therefore, this manuscript reports a modified green synthesis technique of IO NPs in which F. carica fig extract is used as a reducing agent. Additionally, the antifungal activity against Candida and Aspergillus species and antibacterial activity against Gram-positive S. aureus and Gram-negative Acinetobacter species were thoroughly investigated.
Materials and Method

Plant collection and extraction

Dried *F. carica* (common fig) was purchased from local market in Baghdad, Iraq. Consequently, the collected fruit was washed thoroughly; afterward, 10 gm of the washed fruit was blended alongside 150 mL of deionized-distilled water (DDW). The resultant mixture was subjected to heating process at 100 °C in a ventilation oven and then cooled down to room temperature. Finally, the solution mixture was filtered using chromatography paper (Whatman No.1) and incubated at room temperature for further use.

Synthesis of IO NPs

In a typical laboratory route, specific amount (0.1, 0.14 and 0.18 molar) of iron(III) chloride hexahydrate (*F₂Cl₃.6H₂O*) was liquefied in 100 mL of DDW under stirring rate of 800 r.p.m. for 15 minutes at RT. The consequential mixture was then mixed with fig extract (1:1) under stirring rate of 600 r.p.m. for 2 h at 50 °C (1). The first indication of the NPs occurring was observed upon color changing of the solution, whereby a light brown color was noticed, as presented in figure (1). The acquired solution was then washed and centrifuged at 4000 r.p.m. for 20 minutes. Hereinafter, the attained residual was dried for 6 h at 60 °C and later grinded using mortar and pestle to obtain a fine NPs powder.

Figure (1): Color changing process of IO NPs.

Characterization

The microstructural properties of the prepared NPs were examined using Shimadzu X-ray Diffractometer (XRD-6000) with wavelength of 1.541 Å and Cu-Kα radiation. In the meanwhile, compact char surface was investigated using Fourier Transform Infrared Spectroscopy (FT-IR, Thermo Nicolet Nexus) ranging from 400 to 4000 cm⁻¹. Furthermore, the optical properties of the prepared NPs were recorded on Shimadzu UV-1800 UV-Vis spectrophotometer. Atomic Force Microscopy (SPM AA3000-AFM) and TECNAI F-30 TEM were engaged for the morphological and nanoparticle size investigations.

Evaluation of antifungal and antibacterial activities

The antifungal activity of the synthesized NPs was monitored using agar well diffusion procedure against two fungal species which are *Candida* and *Aspergillus* species (22). Agar petri dish, which was used in this study as a culture media holder, was systematically swabbed with sterile cotton swab in which a 30 ml of 24 h Sabouraud’s dextrose was used for each fungal species. Continuously, wells were made in the pre-solidified agar plates with the help of 5 mm cork bor-er. Variety of the synthesized NPs concentrations (0.75, 1.5, 3, 6, 12 and 24 mg/ml) were sonicated with DDW and then used to evaluate the antifungal activity. Concurrently, Negative and positive control against the fungal pathogens was exhibited using DDW and antibiotics. Hereinafter, the cultured agar plates were incubated at 35 °C for 48 h, while the inhibition zones were measured in millimeter.

As for the IO NPs antibacterial activity, similar route to the aforementioned antifungal was repeated. However, the antibacterial activity was screened against Gram-positive *S. aureus* and Gram-negative *Acinetobacter* species using 30 ml of 24 h Blood agar culture media. In
this experiment, the positive and negative controls used were antibiotics and DDW and the antibacterial activity was later proceeded to an incubation procedure for 24 h at 35°C. It is worth mentioning that the concentrations of IO NPs utilized for the antibacterial activity are 0.5, 1, 2, 4, and 8 mg/ml.

**Results and Discussion**

The XRD patterns of the synthesized NPs are presented in figure (2, a). Generally, the intensity of the diffraction peaks augmented with increasing IO NPs concentrations which in turn indicates higher crystallinity at high concentrations. As depicted in the figure, eight different pronounced peaks were acquired corresponding to (110), (120), (211), (10-1), (202), (211), (312), and (310) planes and diffraction angle of 2θ = 21.9°, 26.6°, 33.2°, 35.6°, 49.5°, 50.4°, 54°, and 62.4°, respectively (Card No. 96-901-1413) (1). The (110), (120), and (211) planes mainly belong to FeO phase, while other planes, (211), (10-1), (202), (312), and (310), are corresponded to Fe₂O₃ phase (Card No. 96-900-9783) (9).

Figure (2, b) illustrates the FT-IR curve of the synthesized NPs with concentration of 0.14 M. It is clear to be noticed that characteristic peaks at 3848, 3737, and 3416 cm⁻¹ are mainly attributed to the O-H stretching bonds. In the meanwhile, peak at around 2925, cm⁻¹ is corresponded to C-H stretching characteristic. Two pronounced peaks at 2381, and 2310 cm⁻¹ are designated to O=C=O stretching. Furthermore, additional peaks at 1737, and 1638 cm⁻¹ were observed which are due to C=O stretching, while peak at 1542 cm-1 is found to be in accordance with C=C stretching (1, 23). Finally, Fe-O vibrations namely 1098, 795, 506 and 463 cm⁻¹ are assigned to the IO NPs (23).

The UV-vis spectra of the synthesized IO NPs are demonstrated in figure (2, c), which exhibited broad bands and cut-off phenomenon at 360 nm. Furthermore, as illustrated in the figure, there is a decrease in the mentioned phenomenon cut-off towards higher wavelength as the concentration enlarged; this could be attributed to the lattice defects in the prepared NPs matrix (9).

Figure (2, d) demonstrates two dimensions and three dimensions AFM images of the synthesized NPs with a concentration of 0.14 M and scanning area of 2 µm. In general, the formed NPs exhibited a vertically aligned NPs with regular spherical shape and homogenous distribution. The average diameter and RMS were found to be 84.4 and 0.64 nm, respectively. In the meanwhile, the average surface roughness was found to be 0.54 nm; this indicates a pronounced rough surface which in turn reveals high electrochemical performance (24).
The TEM image of IO NPs is presented in figure (3, a and b). Generally, the synthesized NPs revealed almost a spherical structure with average diameter of 19 nm. From the figure, it also can be observed that a uniform IO NPs distribution which found to be in good agreement with the AFM findings. Furthermore, figure (3, b) shows the NPs diameter distribution which was found to be in the range of 11-29 nm.

Figure (3): IO NPs with 0.14 M concentration (a) TEM image (b) diameter range distribution.

The antifungal activity of the green synthesized NPs against *Candida* and *Aspergillus* species are shown in figure (4, a). As presented in the figure, an observable inhibition zone increment in both kind of fungi species can be clearly seen as the concentration of the IO NPs increased, this indicates the active role of the synthesized NPs as an antifungal. This can be explained by the superior features of IO NPs such as large surface area, and small particle size as compared to their bulk nature. Although, the inhibition zones in the case of *Candida* demonstrated larger inhibition diameter, lower concentrations of the used NPs were found to be active in the case of *Aspergillus*. The maximum inhibition zone diameters were found by the highest IO NPs concentration 24 (mg/ml) against *Candida* (35 mm) followed by *Aspergillus* (33 mm). In contrast, inhibition zone diameters of 10 and 15 mm against *Candida* and *Aspergillus* with IO NPs concentration of 3 mg/ml, respectively. Similarly, at concentration of 1.5 mg/ml, the antifungal activity was only exhibited against *Aspergillus*. This may be due the differences in the *Candida* species’ cell wall structure which consists of high chitin as a yeast as compared to the moldy in *Aspergillus* species, this in turn leads to different sub-capability of each towards the tested nanoparticles.

Figure (4, b) presents the antibacterial activity of IO NPs against Gram-positive *S. aureus* and Gram-negative *Acinetobacter* species. Generally, increasing the concentration of IO NPs exhibited higher antibacterial activity. This could be attributed to the metallic NPs accumulation in the living cell-membranes which in turn releases cellular compounds as previously reported by other researchers. It is a necessity to be stated that concentration as low as 0.5 mg/ml exhibited null activity against both species. However, higher concentrations of IO NPs displayed advanced inhibition zones in both species cases. Continuously, the acquired inhibition zones against Gram-negative *Acinetobacter* bring about more diameter range as compared to Gram-positive *S. aureus* at all concentrations. Inhibition zones of 25 and 19 mm in diameter against Gram-negative *Acinetobacter* and Gram-positive *S. aureus* were screened with IO NPs concentration of 8 mg/ml, respectively. This may be due to the difference in susceptibilities property of each bacterial species towards IO nanoparticles comes from the differences in the structure of cell wall of each bacteria. In details, the Gram-positive *S. aureus* species has thicker peptidoglycan and thus demonstrate higher resistance in contrast to Gram-negative *Acinetobacter*. As observed in table (1 and 2), the synthesized NPs demonstrated higher antibacterial and antifungal effects than standard antibiotics.
**Table (1): Antibiotics zone of inhibition diameters against fungi.**

<table>
<thead>
<tr>
<th>fungal</th>
<th>inhibition zone (mm)</th>
<th>KCA (10 μg)</th>
<th>NY (100 μg)</th>
<th>AMB (20 μg)</th>
<th>FCN (10 μg)</th>
<th>DDW</th>
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<tr>
<td>candida spp.</td>
<td>34</td>
<td>14</td>
<td>11.4</td>
<td>22</td>
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<tr>
<td>Aspergillus spp.</td>
<td>21</td>
<td>16</td>
<td>12</td>
<td>0</td>
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**Table (2): Antibiotics zone of inhibition against bacteria.**

<table>
<thead>
<tr>
<th>bacterial</th>
<th>inhibition zone (mm)</th>
<th>CRO (30 μg)</th>
<th>AK (30 μg)</th>
<th>SAM (20 μg)</th>
<th>TS (25 μg)</th>
<th>CD (2 μg)</th>
<th>CIP (5 μg)</th>
<th>ATH (15 μg)</th>
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<tr>
<td>S. aureus</td>
<td>26</td>
<td>17.5</td>
<td>14.5</td>
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<td>0</td>
<td>0</td>
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<td></td>
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<tr>
<td>Acinetobacter baumanii</td>
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<td>0</td>
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<td>0</td>
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</tbody>
</table>

**Conclusion**

IO NPs were successfully synthesized using *F. carica* fig extract. Additionally, the microstructural properties of the prepared NPs were illustrated using XRD, FT-IR, UV-vis, AFM and TEM techniques. Simultaneously, the synthesized NPs were evaluated for their antifungal and antibacterial activities against *Candida* and *Aspergillus* species as well as *S. aureus* and *Acinetobacter* species, respectively. It was found that *Aspergillus* species is more sensitive to the synthesized NPs rather than *S. aureus*, shedding the light towards this technique which may be useful for the treatment of this aggressive multidrug resistant bacteria.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq.
Conflict of Interest: Non

Funding: Self-funding

References


Molecular Detection of Antibiotics Resistance Genes in *Burkholderia Cepecia* Isolated From Diabetic Foot Infection

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¹University of Kufa-Faculty of Science-Department of Biology/Iraq

**Summary**

This study aimed to isolate and diagnose *Burkholderia cepacia* from clinical specimens of diabetic foot and study the resistance of bacteria to antimicrobial agent in Najaf governorate from August 2019 to November 2019, which includes 120 clinical specimens for both sexes with an age ranged between (35-70) years old. The diagnosis of bacteria isolates was based on microscopy, as well as the culture and biochemical characteristics as an initial diagnosis. The final diagnosis by the Vitek-2 compact system.

*Burkholderia cepacia* 8 (6.6%). Antibiotic sensitivity test was examined by dick diffusion method, *Burkholderia cepacia* isolates showed high level of resistance almost for all β-lactam antibiotic classes under study which included; ceftriaxone, cefoxitin and Cefepime, with percentage of (100%); ticarcllin with clavulalnic acide, piperacillin, ceftazidimne, tobramycine, ciproflaxcin and levofloxacin with percentage of (87.5%); aztreonam and amikacine with percentage (62.5%); meropenem, imipenem and gentiamycin with percentage of (37.5%).

At molecular study, the investigated the presence of antibiotic-resistant genes (blaImp, blaOxa, blaKpc,blaCTX-M) using PCR technique and electrophoresis systems. 6/8 *Burkholderia cepacia* isolates were with *blaOXA* and (6/8) of isolates carry *blaCTX-M* gene, all isolates of *Burkholderia cepacia* gave negative result of *blaIMP* and *blaKPC* gene. Finally PCR analysis showed that the integron gene was (3/8 %).

**Keyword:** Bcc, *Burkholderia cepacia* ; Antibiotics Resistance; Diabetic

**Introduction**

The *Burkholderia cepacia* complex (Bcc) organisms are opportunistic nosocomial pathogens capable of causing severe disease in immunocompromised individuals. Bacteria frequently employ disparate mechanisms that act synergistically to achieve elevated resistance (1).

However, these data may overestimate the occurrence of resistance in *Burkholderia cepacia* organisms as the study was carried out on patient isolates solicited because they were in fact multidrug resistant. Despite this caveat, resistance patterns, both intrinsic and acquired, must not be discounted in these organisms. The often high-level acquired or intrinsic resistance of non-enteric bacteria such as *P. aeruginosa* and *Burkholderia* species is in no small part attributable to synergy between reduced penetration into and efflux from the cell (2).

Diabetes mellitus (DM) is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Such a deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body’s systems, in particular the blood vessels and nerve and constitutes the most frequent diabetes-related cause of hospitalization (3).

Diabetic foot ulcers is one of the main causes of mortality and morbidity among people with diabetes. Its include an injury to all layers of skin, necrosis or gangrene that usually occur on the soles of the feet, as a result of peripheral neuropathy or peripheral arterial disease (PAD) in diabetes patients (4).

**Materials and Method**

specimens collection and bacterial identification
A total of 120 samples were collected from diabetic foot ulcer who attended different hospitals during the period from August 2019 to November 2019 in Al-Najaf provenance, sample collection include, collection 120 pus samples swab specimens from diabetic foot infection ulcer. The specimens were transported by sterile transport swabs to the department of bacteriology laboratory. Each specimen was inoculated using direct method of inoculation on culture of selective media namely MacConkey, Blood , Mannitol agar then inoculated at 37°C for 18-24 hours.

**DNA Extraction**

Genomic DNA was extracted by using a commercial extraction system (Genomic DNA promega Kit). Molecular Identification

Gel electrophoresis was used for detection of DNA by UV transilluminator. The PCR assay was performed to detect the antibiotic resistance gene for *Burkholderia cepacia* shown in table (2). This primer was designed by Alpha DNA company, Canada as in table (1). Amplified products were confirmed using 1% agarose gel electrophoresis to estimate the PCR products size. The gel was stained with 4 µL of 10mg/mL ethidium bromide (Sigma, USA) and it run at 80V for 1.5h. A single band was observed at the desired position on ultraviolet light transillumintor (Cleaver, UK); bands were photographed using gel documentation system (Cleaver, UK). A 100bp ladder (Bioneer, Korea) was used to measure the molecular weights of amplified products.

**Table (1): Primers used in this study**

<table>
<thead>
<tr>
<th>Primer Type</th>
<th>Primer Target</th>
<th>Primer sequence (5’-3’)</th>
<th>Amplicon size (bp)</th>
<th>Reference</th>
</tr>
</thead>
</table>
| CTX-M       | blaCTX-M      | F: SCS ATG TGC AGY ACC AGT AA  
|              |               | R: CCG CRA TAT GRT TGG TGG TG | 554     | (7)       |
| KPC         | blaKPC        | F: ATG TCA CTG TAT CGC CGT CT  
|              |               | R: TTT TCA GAG CCT TAC TGC CC | 893     | (8)       |
| IMP         | blaIMP        | F: TTGACACTCCATTTACDG  
|              |               | R: GATYGAGAATTAAGCCACYCT | 139     | (9)       |
| OXA         | blaOxa        | F: GGCACCAGATTCAACTTTCAG  
|              |               | R: GACCCCCAAGTTTCTCGTAAGTG | 564     | (9)       |

**Table (2): PCR program of intI primer that apply in the thermocycler**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Initial denaturation</th>
<th>No. of cycles</th>
<th>Denaturation</th>
<th>Annealing</th>
<th>Extension</th>
<th>Final extension</th>
</tr>
</thead>
<tbody>
<tr>
<td>blaCtxm</td>
<td>94 C° for 4min.</td>
<td>35</td>
<td>94 C° for 30Sec</td>
<td>63 C° for 1 min</td>
<td>72 C° for 1min.</td>
<td>72 C° for 5min.</td>
</tr>
<tr>
<td>blaKpc</td>
<td>94 C° for 5 min.</td>
<td>35</td>
<td>94 C° for 1min</td>
<td>50 C° for 1 min</td>
<td>72 C° for 1min.</td>
<td>72 C° for 10min.</td>
</tr>
<tr>
<td>blaImp</td>
<td>94 C° 10 min</td>
<td>30</td>
<td>94 C° for 40Sec</td>
<td>55 C° for 40Sec</td>
<td>72 C° for 1min</td>
<td>72 C° for 10 min</td>
</tr>
<tr>
<td>blaOxa</td>
<td>94 C° for 10 min</td>
<td>30</td>
<td>94 C° for 40Sec</td>
<td>60 C° for 40Sec</td>
<td>72 C° for 1min</td>
<td>72 C° for 5 min</td>
</tr>
</tbody>
</table>
Results and Discussion

This study was conducted on 120 specimens from diabetic foot suspected patients during the period from September 2019 to December 2019.

4.9.1.2 Molecular identification of antimicrobial drug resistance of *Pseudomonas aeruginosa* and *Burkholderia cepacia*

4.9.3.2.1 *blaOXA*

The result showed that the *blaOXA* resistance gene was detected in 6/8 *Burkholderia cepacia* as in figure (1).

![Figure](image)

(1): PCR amplification products of *Burkholderia cepacia* isolates that amplified with *blaOXA* gene primers with product 564bp. Lane (L), DNA molecular size marker (100-bp ladder), Lanes (1,2,3,5,6,7) show positive results with *blaOXA* gene.

Carbapenemases are the main mechanism by which resistance to carbapenems occurs and they belong to three of the four β-lactamase classes A, B and D. Class D carbapenemases are the OXA-β-lactamases, further subdivided into various sub-groups mainly *blaOXA-23, blaOXA-24/40, blaOXA-58, blaOXA-48, blaOXA-51* and *blaOXA-143*. These OXA-type β-lactamases occur widely in Acinetobacter with the most abundant being *blaOXA-51*, which is chromosomally encoded hence intrinsic to these species but it may confer resistance to carbapenems when its expression is up-regulated by genetic re-organization. Class B carbapenemases are also known as the metallo-β-lactamases (MBLs), they are mostly encoded by integronborne mobile gene cassettes and hence, they are transferable amongst various bacteria via horizontal gene transfer mechanisms notably conjugation. Class A carbapenemases include the Klebsiella pneumoniae carbapenemase (KPC) family that can be plasmid encoded or chromosomal.

4.9.3.2.2 *blaIMP*

The result showed negative with *blaIMP* gene of *Burkholderia cepacia* isolates. Based on recent reports, there are two major families of imported metallo-β-lactamases, IMP and VIM, that are carried on mobile gene cassettes inserted into integrons. Including those in this report, there are 18 variants of IMP metallo-β-lactamases and 11 variants of VIM metallo-β-lactamases.

The only two published reports on metallo-B-lactamases from the United States identified VIM-2 and VIM-7. Metallo-B-lactamases hydrolyze most β-lactam antibiotics except aztreonam. Therefore, many pathogens that produce these enzymes at high levels are resistant to the majority of β-lactam antibiotics, including the carbapenems. The first report of an imported metallo-B-lactamase described a *Pseudomonas aeruginosa* isolate obtained from a Japanese patient in 1988.

Since then, the occurrence of mobile genetic elements encoding metallo-B-lactamases has extended beyond *P. aeruginosa* to include many types of gram-negative organisms distributed throughout the world. Areas which have reported these types of isolates include several countries in Asia and Europe; the Americas, including Brazil, Canada, and the United States; and Australia.

4.9.3.2.3 *blaKPC*

All *Burkholderia cepacia* isolates give negative result with *blaKPC* gene.

The *blaKPC* genes that encode KPCs are present on transferable plasmids and are flanked by transposable elements, thus allowing for the gene to move from plasmid to the bacterial chromosome and back. All the carbapenem resistant isolates showed 100%
resistance to ampicillin, cotrimoxazole, all 4 generations of cefalosporins and piperacillin tazobactam. The resistance to aminoglycoside antibiotics varied from 33% for amikacin to 94% to tobramycin. In the present study, *Klebsiella* showed a 77% resistance to imipenem and 96% resistance to meropenem, while *E coli* showed 67% resistance to imipenem and 95% resistance to meropenem, blaKPC gene Detection in Clinical Isolates of Carbapenem Resistant Enterobacteriaceae were MHT negative. They may have developed a different resistant mechanism other than carbapenemase production. Resistant to both imipenem and meropenem is a strong indicator of carbapenemase production rather than resistance to either one of the carbapenems, as this may imply a different resistance mechanism (14).

4.9.3.2.4. **blaCTX-M** gene

The result showed that the blaCTX-M resistance gene was detected in and 6/8 *Burkholderia cepacia* as in figure (2).

Figure (2): PCR amplification products of *Burkholderia cepacia* isolates that amplified with **blaCTX-M** gene primers with product 554bp. Lane (L), DNA molecular size marker (100-bp ladder), Lanes (1,2,3,4,6,7) show positive results with **blaCTX-M** gene.

ESBLs are one of the main leading causes of resistance to β-lactam antibiotics among Gram-negative bacteria. These enzymes are plasmid-encoded β-lactamases that mediate resistance to penicillins, first-, second- and third- generation cephalosporins, such as cefotaxime, ceftriaxone, and ceftazidime. TEM, SHV, and CTX-M are the major genetic groups of ESBLs amongst clinically important Gram-negative bacteria. These enzymes are most commonly found in *Klebsiella pneumoniae* (K. pneumoniae) and Escherichia coli (E. coli) and are also observed in other clinical isolates of Enterobacteriaceae and Pseudomonas (15). The first TEM-type β-lactamase, produced by a clinical E. coli strain, was reported in 1965. The TEM-type ESBLs are derivatives of TEM-1 and TEM-2. The SHV-type ESBLs may be found in clinical isolates more frequently than any other types of ESBLs and have been reported from several countries in Europe, such as Austria, France, Italy, and Greece, as well as in the United States and Australia. The CTX-M-type ESBLs developed from TEM and SHV and can be divided into five subgroups according to their amino acid sequence simi-larities, including CTX-M-I, CTX-M-II, CTX-M-III, CTX-M-IV, and CTX-M-V (16).

**Ethical Clearance**: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest**: Non

**Funding**: Self-funding

**References**


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Evaluation the Antimicrobial Effect of Glycerin Magnesia on Some Bacteria, in-Vitro Study

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Abstract

This study was aimed to assess the efficacy of glycerin magnesia on some bacteria. A thirty percent of glycerin magnesia were prepared as explained below. Many types of bacteria including Proteus spp., Staphylococcus aureus, Staphylococcus epidermidis, Acinetobacter, E. coli, Pseudomonas aeruginosa, Salmonella spp. were selected for this study and obtained from university of Tikrit, college of veterinary medicine. A bacterial broth were prepared, a then a sterile swab were emulsify in these broth and streaked on muller hinton agar plate and allowed till dry, then a holes were filled with a given glycerin magnesia and incubated for 24hrs. at 37°C. The results showed that a higher antibacterial effects of glycerin magnesia against Staphylococcus aureus followed by Proteus spp., Pseudomonas aeruginosa, Salmonella spp., Acinetobacter, E. coli, Staphylococcus epidermidis respectively.

In conclusion, the glycerin magnesia have a wide range antibacterial effect and can be used in future in wound healing.

Key words: glycerin magnesia, bacteria, in-vitro.

Introduction

Magnesium (Mg) is an important ion found in the body human and animals, and may be observed as a drug with numerous clinical uses (1). The total magnesium in human and animal body were 53 % stores in the bones, 27 % in muscle, 19 % in other soft tissues, about 0.5 % in the RBCs and about 0.3 % in the serum (2). About half of Magnesium are existing as free and doesn’t bound with albumin or other ion (3).

Magnesium sulfate (MgSO4) is a solid, odorless material that present as crystal powders or as colorless crystals needle like or as a crystalline powder with white color (4).

Magnesium sulfate have a wide uses in building, industrial/processing, agriculture, special care products, medicine, food processing (5).

Ismail & Shaker (7) reported that MgSO4 with glycerin have an antibacterial effects when used on wound. The addition of glycerin as a vehicle for MgSO4, some reported that glycerin increase the antimicrobial activity of material that emulsify in it (8).

The most pathogenic bacteria for human and animals were reported including Salmonella (9-13), E. coli (14-17), Staphylococcus spp. (18), Proteus spp. (19).

The current study aimed to assess the efficacy of glycerin magnesia against some bacteria.

Materials and Method

Glycerin Magnesia (30%): The mixture was
prepared by dissolved a 30 g of MgSO4 in 30 ml heated distilled water and mixing thoroughly. After a complete dissolving of MgSO4, the glycerin was added slowly to the mixture till a final volume of 100ml and using heat and continual stirring.

Many types of bacteria including Proteus spp., Staphylococcus aureus, Staphylococcus epidermidis, Acinetobacter, E. coli, Pseudomonas aeruginosa, Salmonella spp. were selected for this study and obtained from university of Tikrit, college of veterinary medicine. Each type of these bacteria were inoculated in 5 ml nutrient broth and incubated for 24hrs., then a sterile swab were emulsify in these broth and streaked on muller hinton agar plate with a central hole (which made in each of the plate with a sterile 2.0 mm diameter cork borers) and allowed till dry, then a holes were filled with a given glycerin magnesia and incubated for 24hrs. at 37°C.

Results and Discussion

The current results obtained were showed a higher antibacterial effects of glycerin magnesia against Staphylococcus aureus followed by proteus spp., Pseudomonas aeruginosa, Salmonella spp., Acinitobacter, E. coli, Staphylococcus epidermidis respectively (Table 1) (figures 1, 2).

Table (1) Antibacterial effects of glycerin magnesia on some bacteria.

<table>
<thead>
<tr>
<th>Type of Bacteria</th>
<th>Diameter in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>40</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>5</td>
</tr>
<tr>
<td>E. coli</td>
<td>25</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>30</td>
</tr>
<tr>
<td>Acinitobacter</td>
<td>28</td>
</tr>
<tr>
<td>Proteus spp.</td>
<td>35</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>30</td>
</tr>
</tbody>
</table>

It has been reported that Mg alone has an antibacterial effect invitro by reduction the bacterial growth and prevent the formation of biofilm by increasing of local alkalinity (20). Robinson et al. (21) suggested that the alkaline pH was liable for the significance decreasing of CFU/ml1.

Another researcher reported that the pH and the graded charge were significant in prokaryotic physiology in production the proton motion force that used to do the useful work for bacteria, also reported that a pH is important for extra and intracellular environments favored for growth of organisms (22). Also, other researcher reported that some biomaterial have an ability to yield the alkaline pH which give rise the antibacterial mechanism (23, 24).

Li et al. (20) reported that pure magnesium when added to medium has a full effect on MRSA when tested in-vitro.
Also, Crisler et al. (25) found that the increasing medium salinity to 10% MgSO4 reduced the number of *Pseudomonas*. However, a report of Marnocha et al. (26) suggested that MgSO4 at 10% reduce the growth of most tested bacteria.

Many studies have been suggested that there was an association amongst the cell membrane fluidity and the stress tolerance. The structure of cell membrane fatty acids is liable for preservation of cell membrane fluidity. The most consequences of altering in fatty acid of cell membrane in most microorganisms are inflection the action of cells intrinsic proteins which achieve many purposes for example uptake of nutrient and ion pumping (27). The treatment by using alkaline material resulted in alterations in fatty acid composition of the cell membrane for some bacteria especially *E. coli* and *Salmonella Spp.* which leading to reduction in number of these bacterial types (28).

**Conclusion**

Glycerin magnesia have a wide range antibacterial effect on many bacteria especially *S. aureus*, *Pseudomonas* and other, the future study will use this preparation in wound healing.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest:** Non

**Funding:** Self-funding

**References**


The Role of CTX-II, Dyslipidemia, Vitamin D in Polycystic Ovary Syndrome

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Abstract

This study aimed to study the serum measurement of clinical value of type II collagen (CTX-II) in syndrome of polycystic ovary in women and indicter as osteoarthritis disease marker analyzed in future levels with a recent available immune enzyme-linked sorbent assay (ELISA) kit. For increasing sensitivity test, the protocol was modified. Levels of CTX-II increased significantly as well total protein, while decrease vitamin D and difference in lipid profile were practical between the patients compared with healthy women’s. The obtained results suggested the monitoring interest of the serum CTX-II for the OA development in patients of polycystic ovary syndrome in women and the relevance of the analysis of multiple time point for this biomarker.

Keyword: CTX-II- Biomarkers- OA Osteoarthritis- Polycystic ovary syndrome- Dyslipidemia.

Introduction

Syndrome of polycystic ovary (PCOS) is a hard condition with characteristics of prominent levels of androgen, irregularities of menstrual and/or small cysts on either ovaries or both (1). This disorder might be of ovaries morphological polycystic or hyper androgenemia predominantly biochemical. Hyper androgenism, is a PCOS clinical hallmark result of inhibition of follicular progression, ovaries microcysts, , changes in menstrual and ovulation (2). Investigations proposed that (5% to 10%) females of age 18 - 44 years are PCOS affected, rendering it the most well-known abnormality of endocrine among females of reproductive age in U.S.A. (3). Women looking for assistance from professionals in health care to address issues of excessive hair growth, obesity, acne, infertility, and amenorrhea most of the times receive a PCOS diagnosis and have cancer of endometrial at higher rates, TIIDM, dyslipidemia and cardiovascular disease (4).

PCOS pathophysiology involves defects primary in insulin secretion and action, ovarian function, and the hypothalamic–pituitary axis. Although the unknown cause of PCOS, PCOS has been connected to obesity and insulin resistance (5). There are 3 most well-known elements with PCOS associated i.e. irregularities of ovulation, androgen levels enhancement, and problems of cystic ovaries with elevated androgen levels and ovulation take place in most PCOS women (24, 26). Furthermore, alopecia, hirsutism, and acne are directly associated with elevation levels of androgen and the ovaries prevalence of polycystic on pelvic ultrasound more than 70% in PCOS patients (6).

After diagnosing PCOS, investigations prove that patients over 50% have diabetes or pre-diabetes, and there is risk increasing of hypertension, myocardial infarction (MI), dyslipidemia, osteoarthritis sleep apnea, anxiety, depression, and endometrial cancer. Furthermore, PCOS pregnant women should be noticed for increasing miscarriage rates, pre-eclampsia, premature delivery, and gestational diabetes (7). Osteoarthritis defined as a disease with developing articulate cartilage destruction and by changes pathologically in the subchondral bone and synovial membrane OA, the destruction will result in losing the 2 major components, type II collagen and proteoglycans, rendering them choice markers in determining metabolism of cartilage (8). Peptide of C-telo of collagen type II (CTX-II) is marker most studied (9). Increasing levels were documented in OA patients.
in comparison to subjects of asymptomatic or without OA signs (10). A significant association have shown between CTXII concentration and OA radiographic development (11). The goal of this investigation was to study the serum measurement of clinical value of CTX-II in polycystic ovary syndrome patients and correlated with physiological assessment that give indicator to development of Osteoarthritis in future.

**Methodology**

Serum specimen was collected from patient with infertile Polycystic Ovary patients (n = 45) and healthy patient (n=45) at AL-Sader laboratory of Medical city in Najaf Province, AL-Najaf Health Directorate / Ministry of Health /Iraq. The average of the patient’s age was (32.81±51) years. All reagents and specimens should be at a temperature of room before use. Reagents mixed in soft way with no foaming. No interruption should take place once the protocol started Tests of biochemical were performed at Biology laboratories. in this study was CTX-II protein (MBS2507692), vitamin D3 (MBS773966) , HDL (MBS170439) , VLDL(MBS265004), total protein (MBS2540455) and LDL (MBS162140) My Bio Source Company USA in Origin.

**Statistical Analysis**

Statistical analyses of all result were carried out by the help of Graphpad prism version 5) software statistical package using t-test (with p value at level of significant less than 0.05) to compare values of result between groups.

**Results**

The result show an increase of significant for protein of collagen matrix Type II figure( 1) in patient with Polycystic Ovary(mean± Std. Error 3.98±0.67) compare control group (mean± Std. Error 2.62±0.71) which that may be cause by the extracellular adhesion molecule is soluble from the cartilage surface component. Enable the binding of cartilage cells to type II collagen in the absence of serum, thereby increasing protein externally, we conclude that direct interaction between cartilage cells and type II collagen occurs through other adhesion mechanisms of cell surface proteoglycans such as membrane-bound heparin sulfate in progressive age (12). Few studies revealed a significant relation between concentration of CTXII and OA radiographic development (13). Recently, urinary levels predictive value of CTX-II for cartilage losing was assessed through MRI (14).

![Figure 1: levels of Type II protein collagen matrix protein in patients comparison to Healthy group](image)

These results was show a decrease of vitamin D3 in patient with Polycystic Ovary (mean± Std. Error 12.5±0.17) compare control group (mean± Std. Error 24.4±0.91) figure (2), Probably Vitamin D deficiency may be associated with populations with extensive skin coverage, especially in women in Iraq. There is a large body of proof the importance of vitamin D in reproductive function because VDRs have been detected in placenta, the endometrium and ovary (15). Deficiency of Vitamin D is associated with deregulation of Ca, which participates in the follicular arrest development in PCOS women resulting in dysfunction fertility and menstrual (16). Some study on PCOS women with and undergoing fertilization in vitro (IVF), they found that the women who achieved pregnancy exhibited significantly higher levels of follicular fluid of 25(OH) D and each ng/ml elevate in follicular fluid 25(OH)D elevated the likelihood for pregnancy achievement by 7% (17). Moreover, deficiency of 25(OH)D was related with rates of lower development for pregnancy and follicle after clomiphene-citrate stimulation in PCOS women, suggesting a possible vitamin D supplementation role in PCOS infertile women who undergo stimulation of ovarian (18).
The results revealed an increasing levels of significant in LDL level in patient with polycystic (mean± Std. Error 118±0.64) ; control group (mean± Std. Error 44.4±0.26) figure(4), also The result show decrease significant level in HDL level in patient polycystic (mean± Std. Error 39.4±1.14) ; control group (mean± Std. Error 49.4±2.41) figure (5),suggests that the level of LDL, HDL, insulin, glucose, apolipo protein A1, and lipoprotein a, in female with and without PCOS do not show any significant differences (20). Similarly, Jahanfar et al., in a study aimed at evaluating the genetic and environmental factors affecting lipids among twins, found no significant difference (21). The research concluded that the serum CTX-II in women with PCOS may appear to have symptoms of Osteoarthritis and thus it is considered to be a vital reliable evidence in the news of the occurrence of this disease in PCOS women.
Results proved a significant enhancement of VLDL level in patient (mean± Std. Error 29±2.34) ; control group (mean± Std. Error 20±2.64) figure(6) . Disturbances in metabolic are famous clinical syndrome characters, especially, dyslipidemia which is very well-known abnormality metabolic in PCOS female with a prevalence of up to 70% (22,23). Resistance of insulin is a key PCOS pathophysiology and dyslipidemia in PCOS women may be therefore in accordance with that detected in the insulin resistant case: decreasing levels of apolipoprotein (Apo) A-I, and high-density lipoprotein-cholesterol (HDL-C), and increased levels of ApoB, triglycerides (TG) and very low-density lipoprotein(24, 25, 26).

**Figure 6: levels of VLDL in patients comparison to Healthy group**

**Conclusion**

The research has a goal of examining the clinical value of the serum which was measured type II collagen (CTX-II) in the Polycystic ovary syndrome in the women and indictor as a marker of the (OA)disease of future levels. An important percentage increased in CTX-II levels, and the total protein but a decrease occurred in vitamin D and with a change in lipid profile which was in practical status between the patients compared with healthy women.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest:** Non

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**References**


Prevalence of Histopathological Diagnosis of Benign and Malignant Breast Lesions in Al-Muthanna Province for Two Years Duration

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Abstract

Introduction: Breast cancer (BC) is a common form of cancer among women globally. It is considered 5th cause of death in females with equally to 522,000 patients / year (1). Nowadays it is considered 15.4% of death in developed countries after lung cancer. In developing nations, breast cancer considered the 1st cause of death for females 324,000 cases of deaths (14.3%) for total deaths. Method: One hundred and six female patients with breast lesion were collected from the Al-Hussein Teaching Hospital Laboratory/Histopathology department –Al-Muthanna province, during the period from January 2018 to January 2020. The data for cases were collected to study the age, type of breast lesion whether benign or malignant. Haematoxylin/Eosin staining done in Al-Hussein Teaching Hospital Laboratory / Histopathology department. Results: Cross sectional study for 106 patients done in Al- Muthanna proven for assessment breast mass and identify malignant and benign lesion, mean age of patients was (39.4 ± 15.8) years old, with min age 13 years and max age was 81 years old. Types of biopsy taken from surgeon were 71% excisional biopsy, 21% mastectomy and 8% true cut biopsy. After pathological assessment of biopsies showed 51% malignant and 49% benign. In addition, this assessment distributed as following: fibroadenoma 31.1%, IDC/ grade II 29.2%, IDC/ grade III 9.4%, ILC and fibrocystic changes 5.7% and IDC grade I 3.8% and other types of malignant and benign after pathological assessment. Significant association between age groups and cancer of breast. Conclusion: After pathological assessment of biopsies, malignant breast cancer (IDC/ grade II, IDC/ grade III, ILC and fibrocystic changes) most common changes, significant association between age group and breast cancer development more age group 41- 50 years old and then (31– 40), (51– 60) years old respectively.

Key words: Prevalence, histopathological diagnosis, breast lesions, Al-muthanna province

Introduction

Breast cancer (BC) is a common form of cancer among women globally. It is considered 5th cause of death in females with equally to 522,000 patients / year (1). Nowadays it is considered 15.4% of death in developed countries after lung cancer (2). In developing nations, breast cancer considered the 1st cause of death for females 324,000 cases of deaths (14.3%) for total deaths (2,3). This rate changes from 6 – 20 / 100.000 in West Africa and East Asia (4). The incidence in 2012 in females reach to 1.7 million (25% of entirely cancers), 883,000 patients in developed country in contradiction of 794,000 in developing nations (4,5).

The incidence was increase in age after 35 years old and peaking in 60 years old (6).

Mortality rate depend on age of females, staging of malignancy, treatment respond, metastasis of malignancy, main reasons of breast cancer is hormonal factors, genetic tendencies, behaviors and ecological reasons (7).
Benign Breast Diseases (BBDs) is not cancer. Most usual reason of breast difficulty in women and more usual than malignancy (8, 9). It is more usual than malignancy in western countries (10). More than 30% of women with BBD need treatment where survive (11). A three-ways for evaluation done by clinical assessment: US and mammogram, pathological assessment, central needle biopsy. Most women with BBD not have risk of development of breast cancer, so early diagnosis and treatment is important to remove the anxiety especially women with family history of breast cancer and must follow up annually. Most common BBD is fibro adenoma (12). Two types of breast cancer (cancer in situ): Ductal carcinoma in situ. (DCIS) 83% of all cases 2010-2014 unusual cells substitute usual epithelial cells around ducts of breast and metastasis to the lobules in addition to the ducts; it is can or can not developed to the aggressive malignancy it is grow slowly without any management it is misdiagnosis with benign 20%-53% diagnosed with an invasive breast malignance for 10 years or more. Lobular carcinoma in situ.: 13 % of patients, atypical cells developing inside and growing about some of breast lobules. It is not precursor of invasive cancer, but considered one of strong cause of invasive cancer. The aim of study is to show proportion of benign and malignant breast lesions and types of malignancy tissues by pathological assessment and relation of malignancy with increase age of females (13-15).

**Method**

One hundred and six female patients with breast lesion were collected from the Al-Hussein Teaching Hospital Laboratory/Histopathology department –Al-Muthanna province, during the period from January 2018 to January 2020. The data for cases were collected to study the age, type of breast lesion whether benign or malignant. Haematoxylin/Eosin staining done in Al-Hussein Teaching Hospital Laboratory / Histopathology department.

**Specimens:**

The one hundred and six cases classified into benign and malignant breast lesions. From each formalin fixed paraffin embedded tissue, one section of 5-micron thickness was obtained and stained by haematoxylin and eosin staining method for evaluation of morphology.

Methods of staining procedures:

The following steps were applied for (H&E) staining method.

a) Deparaffinization: This done by adding the following:

1. 5 min. period adding Xylene.
2. 5 min. period adding Xylene.
3. 5 min. period adding 99 % ethanol
4. 5 min. period adding 99 % ethanol
5. 5 min. period adding 99 % ethanol
6. 5 min. period adding 99 % ethanol
7. 5 min. period adding 99 % ethanol
8. 5 min. period adding 95 % ethanol
9. 5 min. period adding 70 % ethanol
10. Purified water.

b) Hematoxyline and eosin staining method:

1. Dewax sections (deparaffinization as above).
2. Stain in hematoxyline for 3-10 minutes.
3. Wash well in running tap water.
4. Remove excess stain by differentiating the sections in 1% acid alcohol (1% in HCL 70% alcohol) for 5-10 seconds.
5. Wash well with in tap water until sections regain their blue color.
6. Stain in eosin for 2-5 minutes.
7. Dehydrate slowly through increasing grades of alcohol (i.e.70%, 90% and 100%).
8. Clearing by xylene.
9. Mount wit DPX.

Statistical analysis done by SPSS 22 calculated mean and SD with percentage and frequency. Chi square use for revealed association between age groups and behavior of tumor, significant association when P-value less than 0.05.

**Results**

Cross sectional study for 106 patients done in Al-
Muthanna proven for assessment breast mass and identify malignant and benign lesion, mean age of patients was 
(39.4 ± 15.8) years old, with min age 13 years and max age was 81 years old. Types of biopsy taken from surgeon 
were 71% excisional biopsy, 21% mastectomy and 8% true cut biopsy as showed in fig (1). After pathological 
assessment of biopsies showed 51% malignant and 49% benign as in fig (2). In addition, this assessment distributed 
as following: fibroadenoma 31.1%, IDC/ grade II 29.2%, IDC/ grade III 9.4%, ILC and fibrocystic changes 5.7% and 
IDC grade I 3.8% and other types of malignant and benign after pathological assessment showed in fig (3).

Fig (1): distribution of types of biopsy.

Fig (2): distribution of behavior of biopsy after pathological assessment.
According to association between ages groups and behavior of lesion either benign or malignant, there is significant association between age groups and cancer of breast, 37% of females in age group 41-50 years old with malignant breast cancer, while 20% of malignancy in age group (31-40) and (51-60) years old. 13% of malignancy in age group 61-70 years old and only 6% in female over 71 years old age. As show in table (1).

<table>
<thead>
<tr>
<th>Age</th>
<th>Count (malignant)</th>
<th>Count (benign)</th>
<th>% within behavior (malignant)</th>
<th>% within behavior (benign)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>11 - 20 years</td>
<td>13</td>
<td>0</td>
<td>25.0%</td>
<td>0.0%</td>
<td>12.3%</td>
</tr>
<tr>
<td>21 - 30 years</td>
<td>22</td>
<td>2</td>
<td>42.3%</td>
<td>3.7%</td>
<td>22.6%</td>
</tr>
<tr>
<td>31 - 40 years</td>
<td>11</td>
<td>11</td>
<td>21.2%</td>
<td>20.4%</td>
<td>20.8%</td>
</tr>
<tr>
<td>41 - 50 years</td>
<td>6</td>
<td>20</td>
<td>11.5%</td>
<td>37.0%</td>
<td>24.5%</td>
</tr>
<tr>
<td>51 - 60 years</td>
<td>0</td>
<td>11</td>
<td>0.0%</td>
<td>20.4%</td>
<td>10.4%</td>
</tr>
<tr>
<td>61 - 70 years</td>
<td>0</td>
<td>7</td>
<td>0.0%</td>
<td>13.0%</td>
<td>6.6%</td>
</tr>
<tr>
<td>more than 71 years</td>
<td>0</td>
<td>3</td>
<td>0.0%</td>
<td>5.6%</td>
<td>2.8%</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>54</td>
<td></td>
<td></td>
<td>106</td>
</tr>
</tbody>
</table>

Pearson Chi-Square = 58.188, P-value = 0.0001** (significant).
Discussion

Cancer is illnesses that lead the cells in body loss of control and change. All types of cancer cells finally changes to lump or mass that named cancer, and this malignant tumor called according to the site form it the tumor originate. Breast cancer start from gland of breast tissue that lead to production of milk called (terminal duct lobular unit). Other parts of breast tissue consist of connective, fatty and lymphatic tissues (16).

According to our study, mean age of patients was (39.4 ± 15.8) years old, with min. age 13 years and max. age was 81 years old. These results similar to study done by Augustin et al. showed the age extended since 16 - 90 years by a mean of 45.83± 13.5 years old (17). Zhi-Gang Yu et.al. Also, have the same results that mean age of patients was (44 ± 11.6) years old (18)characteristics and related factors of breast cancer among women in Eastern China. A total of 122,058 female subjects completed the study, with 320 confirmed cases of breast cancer (crude prevalence: 262.5/100,000; standardized prevalence: 207.7/100,000.

In our study the types of biopsy taken from surgeon were 71% excisional biopsy, 21% mastectomy and 8% true cut biopsy this is similar to Augustin et al. that showed, the total of cases that take and examine by pathologist contain of 69% of lumpectomy then 30% biopsy of breast.

After pathological assessment of biopsies in our study showed 51% malignant and 49% benign while in another study the proportion was around 4:1 or 5:1 (19), this different is due to sample collection or may be due to high no. of malignancy of breast in al-muthanna province.

In addition, this assessment distributed as following: fibroadenoma 31.1%, IDC/ grade II 29.2%, IDC/ grade III 9.4%, ILC and fibrocystic changes 5.7% and IDC grade I 3.8% and other types of malignant and benign after pathological assessment this results similar to Augustin et al. that showed he predominant tumor histological designs IDC 113 (64.9%), ILC 17 (9.8%) then histological designs were invasive ductal carcinoma 113 (64.9%), invasive lobular carcinoma 17 ductal carcinoma in situ 10 (5.7%) and medullary ca. 5 (2.8%) (17). Similar features diagnosed by Ohene et al. (20); detailed that 53.7% of grade III, 31.5% of grade II and 14.8% of grade I. Essiben et al. showed a propensity in the following course of incidence: grades II, I besides III to the Yaoundé Gynecological Obstetric and Pediatric Hospital (21)which is a referral centre for gynecological malignancies. METHODS: It was a retrospective descriptive study over a period of four years, from June 1(st. Koffi et al. presented that tumor grade II exemplified 58.4% of females, however grade I in addition II were in the comparable amount (20.8%) (22)which is a referral centre for gynecological malignancies. METHODS: It was a retrospective descriptive study over a period of
four years, from June 1st. These results are conflicting to those reported by Meye et al. that, the grade II detained the past location after grade I and III (23) which is a referral centre for gynecological malignancies.

METHODS: It was a retrospective descriptive study over a period of four years, from June 1st.

According to association between ages groups and behavior of lesion either benign or malignant, there is significant association between age groups and cancer of breast, 37% of females in age group 41-50 years old with malignant breast cancer, while 20% of malignancy in age group (31-40) and (51-60) years old. 13% of malignancy in age group 61-70 years old and only 6% in female over 71 years old age. Augustin et al. stated the same results that most patients in ages of 35 to 44 and 45 to 54 with 28.2% and 29.3% correspondingly. We can roughly that the major people found is dependable with some preceding studies approved globally (24, 25).

Another study done by Hai-long Chen et al. showed that age groups from 50-59 years old was most group with breast cancer (26.4%), less than 30 years old and less than 40 years old represented low mount of patients (6.4%) (26). In another study stated that 6.4% of patients with breast cancer younger than 40 years old while 93.6% of them more than 40 years old, so the incidence of females to developed breast ca. was more in age group more than 40 years old so all women must do routinely breast cancer screening (27).

Conclusion

After pathological assessment of biopsies, malignant breast cancer (IDC/ grade II, IDC/ grade III, ILC and fibrocystic changes) most common changes, significant association between age group and breast cancer development more age group 41-50 years old and then (31-40), (51-60) years old respectively.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

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A Histological and Histochemical Study on Olfactory Bulbs to Detection Amyloid Protein Depositions by Congo-Red and Routine Staining Techniques

Rajaa Ali Moheiseen Al- Taei, Muna Hussain Al-Aameli, Yarub Modhar Al-Qazwini

Abstract

In this study, histological and histochemical techniques were used to examine olfactory bulbs in the albino rat male. Thirty male albino rats were split into three age groups (10 animals each) in the current research: Group I: consider as a control group, including adult animals aged 3 months. Group II: include animals aged 6 months. Group III: include animals aged 12 months. The histological architecture of the layers of olfactory bulbs and their main cells was identified by using H & E staining techniques, meanwhile the composition of each layer in albino rat was evident. In the glomerular and mitral cell layer of group III, olfactory bulbs showed reduced neural density. Modified staining with Congo - red was conducted for histochemical studies. Compacted amyloid cores were found in group III animals’ olfactory bulbs, while dispersed amyloid cores were found in group II&III olfactory bulbs’ cortex. The present study adds to our knowledge of the impact of amyloid protein on olfactory bulbs and their prospective neurodegeneration involvement.

Key words: Histology, Histochemistry, Congo red, Amyloid, Granular cells, Mitral cells and olfactory bulbs.

Introduction

In the olfactory signal conductive path, the olfactory bulbs are the station of relay. The OBs are the first chief relay in the processing of odor data process: it provides afferent input from olfactory sensory neurons (OSN) in the olfactory epithelium of the nasal cavity and responsible for the identification of odors in it: which is the only relay between the peripheral and central nervous system, it also processed the olfactory data. The olfactory bulb’s main histological architecture involve: olfactory nerve layer, glomerular olfactory layer, external plexiform layer, mitral cell layer, inner plexiform layer and granular cell layer. The superficial olfactory nerve layer contains afferent axons from OSNs. OSN axons synapse with OB projections of neurons dendrites, mitral / cells, and sub aggregations of regional periglomerular cells (Pgm). These layers are arranged in the olfactory bulbs very obviously and regularly. The characteristics of olfactory bulbs structure facilitate the processing of data and also provide a scaffold for olfactory data spatial encoding.

The olfactory bulb consists of four types of cells: mitral cells, granular cells, tufted cells and short axon cells according to classical studies. One of these neurons, granular cells, have long been known to be morphologically uncommon because they don’t have a standard axons, and recent studies in electron microscope have demonstrated that it participate in special reciprocal synaptic connections with mitral cells. Mitral cells are the biggest cells in the olfactory bulbs and as stated by electron and light microscope researches, moreover they are the main olfactory bulb efferent neuron. The dendritic structures of mitral cells can be subdivided into primary and secondary dendrites, and both primary and secondary smooth dendrites pass through the surface of the outer plexiform layer and only the primary dendrites extend down to the olfactory glomerular layer. Within the glomerular layer the dendrites of mitral cells are in synaptic communication with olfactory nerves and periglomerular cells, but the only synapses on mitral cells elsewhere are the “reciprocal synapses” with the granule cells. Mitral cells, as the main olfactory bulbs efferent neurons, play a significant role in olfactory signal conduction and modification.
Amyloids β (AB) are little pieces of a bigger proteins named “amyloid precursors protein” APP. While the ordinary function of APP has not yet been determined by researchers, they understand a lot about how it appears to operate (16). In its full shape APP ranges from the inside of the brain cells to the outside through the fatty membrane around the cells. As APP is activated to perform its normal activities, other protein can cut it into different and smaller parts inside and outside cells. APP can be cut in several aspects: under some conditions, β-amyloid is one of the parts generated (3).

According to the hypothesis of amyloid, phases of beta-amyloid aggregations interrupt cell-to-cell communication and activate immune cells, these cells of immune system cause inflammation (12).

The process of neurodegeneration in diseases of cognition (Alzheimer’s and Parkinson’s diseases) may involve toxicity of β-amyloid (Aβ) that could be demonstrated in vitro and seems to be involving oxidative stress, this underlie the progression of neurodegeneration that consider as characteristic feature of AD (2).

It has been shown that the amyloid induces neuronal death, reduced plasticity of synapses, aberrant axons growth, tau hyper phosphorylation and chronic inflammation (7). Aβ accumulation in the pathogenesis of AD is an early and essential case. First formation of temporal cortical regions, including the hippocampus, a memory-creating zone. Aβ aggregates have been indicated to form neurotoxic plaques that contribute to neurodegeneration accompanied by dementia (15).

Similarly, (11) showed that capillaries, venioles and arterioles in the cerebral cortex also often have amyloid deposits. Congo red staining was considered as an approved histochemical marker for amyloid β-pleated-sheet (14). Congo red: amyloid detection in tissue parts is significantly improved and verified by favorable Congo red staining. Thioflavin S and Congo red are represented the main histological stains that used for any type of amyloid (17).

Red stain is red-pink on its own. Under both light and polarized light microscopy, examination of tissue segments suspected of participation by amyloidosis must be carried out. Amyloidosis has a distinctive green apple birefringence when polarized (11).

In this study we analyzed the histological structure and histochemical (Congo red for amyloid protein) characteristics of OBs in order to evaluate the presence of amyloid depositions in the olfactory bulb layers. The aim of this research is to clarify the amyloid-histochemical and histological characteristics of olfactory bulbs in the rats of albino male in relation with aging.

Material and Method

Experimental Animals:

Male albino rats aged between (3, 6, 12) months were obtained from the Animal House, Collage of Science, University of Babylon. The rats were housed in wire mess cages under standard condition with 12 hrs. Light and 12 hrs. dark cycle throughout the entire experimental period. Food and tap water supplied with libitum.

In the current research, thirty male albino rats will be split into three age groups (10 animals each), they are: Group I: considered as a control group, including adult animals aged 3 months. Group II: include animals aged 6 months. Group III: include animals aged 12 months.

Histological study:

The specimens of olfactory bulbs were taken from the brain of the albino rats, the samples were immersed in the solution of Bouins for two days. Tissue was dehydrated in graded ethanol and embedded in paraffin. 7μm horizontal sections of paraffin blocks were cut on a revolving microtome and installed on glass slides then, the following staining processes were completed (1).

Histochemical Study:

Selected sections have also been processed for histochemical amyloid protein demonstration. Sections have been deparaffinized through xylene and alcohol into tap water. Thereafter, slides are immersed in alkaline sodium chloride. Twenty minutes later, they were immersed in alkaline solution of Congo red and then marked with alcoholic potassium. Thereafter, slides are countered with alum hematoxylin and dehydrated by xylene and ethanol (6).

Results

Histological Study:

In light microscopy, the main olfactory bulbs of the adult rat consisted of six concentrated layers: 1-The olfactory nerve layer (ONL), (fig.1A, and 1B). 2- The glomerular layer (GML). There were observation of
periglomerular cell (Pgm) around the glomeruli (fig.1D, 1E). 3- The layer of the external plexiform layer (EPL), consists of fine nerve fibers and few granule cells, (fig.1A, 1B). 4- A layer of mitral cells (MCL) contained stomata of mitral cells in single row (fig.1A, 1B). They had an ovoid nuclei with single nucleolus deeply stained. Their cytoplasm include darkly stained basophilic granules (fig. 1C, 1E). 5-The inner plexiform layer (IPL), a thin layer of fine nerve fibers and some cells of granules (fig. 1A, 1B). 6- The layer of granule cells (GCL), contained a large amount of granule cells (fig. 1A, 1B).

Fig.1: photomicrograph of histological section demonstrated to the concentric laminar organization of olfactory bulbs, H&E staining.4x, 10x, 40x.

The olfactory nerve fibers have been loosely segregated in group III rats. With a marked decrease in size, glomeruli were distorted in form (fig. 1). The EPL included a number of misplaced mitral cells’ soma (fig.1B, 1E). Most mitral cells in MCL were decreased in size and generally present in a rounded, darkly stained nuclei with undefined nucleolus (fig.1C).

Histochemical Study:

Two forms of plaque of amyloid protein deposition were seen in the cortex of olfactory bulbs: diffuse plaques were shown in all layers of OBs of group III (fig. 2B). Compact amyloid plaques were found in the ONL, GML, MCL, and EPL layers of OBs of group III (fig. 2A, 2B). Amyloid angiophathy were detected in the different layers of OBs of group II and III, (fig.3).

Fig.2: Photomicrograph of histological section showing plaque & diffuse staining for amyloid (AB, blue row) with Congo red–stain in olfactory tissue (2A, 2B), 10 x. Fig.3 (A,B): Photomicrograph of histological section illustrated the Congo red stain highlighted the vascular amyloid depositions, note the staining of vessels walls (black & blue arrow), 40 x.
Fig.3 (A,B): Photomicrograph of histological section illustrated the Congo red stain highlighted the vascular amyloid depositions, note the staining of vessels walls (black & blue arrow), 40 x.

Discussion

In the pathway of the olfactory system, the olfactory bulbs are the essential components of olfactory system and relay station. We studied the histological architecture of the olfactory bulbs, according to the outcomes of our research, surveys were done to the structure of each layer of bulbs. The histological characteristics features of OBs constitute with the prior studies by Golgi (10). Many periglomerular (Pgm) cells were found in the layer of glomeruli of olfactory bulbs. Two groups of neuronal cells are dispersed in the layer of mitral cells of olfactory bulbs, the cells with large cell bodies and cells with small cell bodies. The cells that characterized by large cell bodies seem to be correspond to the mitral cells as output neuronal cells, and the cells with small cell bodies may relate to the tufted cells, that are well known as other kind of neurons of OBs of mammals, or the interneurons in the layer of mitral cells, but the correspondence could not be determined (5).

According to the finding of the present work, the histological characters among the bulbs of olfactory of albino rat are comparable in all groups (I, II, III), but the density of cells in each group was different. The complexity of the olfactory bulbs layers organization proportional to the olfactory bulbs information-processing capacity and represents the degree of olfactory bulb development. The results of our outcomes showed that the morphology and amount of mitral cells in the group I &III were differed (18).

The results of this research are complementary and consistent with prior human OBs tissue reports that prevalent layers (NFL, GML, EPL, MCL, IPL, and GL) constitute the construction of all layers in the olfactory bulbs of albino rats. There was no distinction in olfactory bulb composition between group (I, II, III), except for cell density variations. In group I, in each layer, the density of cells was higher than in group II and III. No mechanism exited to explain this decline. The amount of granulated cells and mitral cells decreased but increased in size (11; 9). The mitral cells considered as the largest neuronal cells in the olfactory bulbs, have primary and secondary dendrites, these processes oriented vertically or parallel to their soma, glial cells formed these dendrites of mitral cells. Axons of mitral cells converge in bundles of fibers and pass through the layer of granular cells (11).

Demonstrated a “substantial layer-specific loose” of synapses ultra-structurally: synaptic density is decreased in the layer of glomerular cells but not the internal plexiform layer, leading to unbalance in circuitry of OBs. Our findings showed a decrease in GML and MCL density, consistent with (10) findings, these results indicate that decreased afferent synaptic input and local modulatory circuit synapses in OB glomeruli may contribute to particular age related changes in olfactory function.
In the current research, we showed diffusing and plaques of compact amyloid nuclei in the cortex of OBs by using modified Congo red staining. We also showed the enhanced amount of plaques and reduced neuronal populations in group III OBs compared to the olfactory cortex of group I & II, that showed typical dark orange colored patches under light microscope. It is well known that there are plenty of extracellular plaques of amyloid β peptide (Aβ) in the pathological marks of AD in the brain (16). Amyloid has been discovered to be more localized in the neuronal processes in the current research, this finding was in agreement with the outcomes of other researchers who noted that in elderly people with and without Alzheimer’s disease abnormality amyloid accumulate as neuropil threads (11). The method of neurodegeneration in AD may involve toxicity of β-amyloid (Aβ). Aβ’s neurotoxicity can be shown in vitro and seems to involve oxidative stress (2).

Our finding demonstrated that the blood vessels within the cortex of olfactory bulbs also lades depositions of amyloid that constitutes with the results of (14). Congophilic amyloid in blood vessels is called cerebral amyloid antipathy (CAA) (17).

Many researchers have shown that the deposition of Aβ peptide in the cerebral cortex leads in neural and morphological degeneration, cognitive loss, and modulation of enzyme markers such as acetylcholine esterase and choline acetyltransferase; all of which are well-known symptoms of AD (19; 4).

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest:** Non

**Funding:** Self-funding

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The Intraperitoneal-Ketoprofen-Histopathological Induced Alterations in the Wistar Rat Kidneys

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Abstract

The current-focused study was carried out to generate a data profile about expected changes that could be induced in the kidneys of rats due to the use of intraperitoneal ketoprofen (KP). The study involved using 24 adult male Wistar rats sorted randomly into four groups (six animals per group). One group was treated as a control group, C, which was supplied with distilled water (DW) only. The remaining animals were presented as (50KP), (25KP), or (12.5KP) groups that received 50mg/kg.b.w., 25mg/kg.b.w., or 12.5mg/kg.b.w., respectively, of KP. The experiment was continued for 70 days, and the kidney tissue samples were collected from the scarified animals at the end of day 70 of that experiment. The kidney tissues of the 50KP animals revealed dilation of the tubules throughout the outer strip of the outer medulla with necrosis and sloughing of the epithelial cells of the proximal convoluted tubules (PCTs). However, the 25KP group suffered lesser grades of epithelial-cell sloughing of the PCTs with lower levels of dilation of tubules than those recorded in the 50KP group. On the other hand, the kidney tissues of the 12.5KP group showed only dilation in the PCTs. The present experimental data unveil the side effects generated by the use of the intraperitoneal KP in the examined rat kidneys which should be used as a launching set of information for better use or further study this drug and its side effects in human patients.

Keywords: Ketoprofen, renal failure, side effects.

Introduction

KP is an anti-inflammatory, anti-pyretic, analgesic derivative of propionic acid with non-steroidal anti-inflammatory (NSAID) therapeutic properties. KP reduces cyclooxygenase I and II enzyme activity, which contributes to a reduction in prostaglandin and thromboxan precursor production. As a consequence, the decline in prostaglandin production triggers the therapeutic potential of ibuprofen via the enhancement of prostaglandin synthase. The production of A2 thromboxane by thromboxane synthasises, which prevent the accumulation of platelets, is also decreased with KP (1). KP is the 11th most popular in Italy with 206 records for 2008, of which some 30 percent is extreme, based on a survey of random cases of adverse outcomes. A maximum of 13 percent of reported data were in the aspect of pediatric patients (under 18 years of age), even in the age group (under six years old) of off-label medication use. The 2012 evidence is not fully accessible; however, unofficial statistics show that KP was implicated in 560 adverse drug reactions, of which 31 percent were seriously affected (2,3).

KP records are that because of the unwise consumption of KP in the world countries despite multiple warnings about hepatotoxicity compared to other NSAIDs such as nimesulide. The KP's reported adverse events contain peripheral edema as a cardiovascular response, central responses such as drowsiness, headache, etc., dermatological responses including skin sensitiveness and photosensitization, blood based responses e.g. edema, platelet malfunction, etc., kidney, elevation of enzymes in the liver, gastrointestinal reactions for example vomiting, diarrhea, gastric ulcer and bleeding, etc. Additional studies have shown at least intermittent serum aminotransferase elevations ranging between one percent and two percent of the patients receiving KP. Even with product continuity, these can be

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overcome. For less than one percent of cases, reported elevations of aminotransferase (more than threefold increased) exist. It is very unusual that liver damage with KP jaundice is clinically evident and only few cases have been recorded. The latency to onset of the symptoms is a quick process, sometimes appear within a few days. The trends of enzyme changes differ between hepatocellular and cholestatic. In some instances, immunoallergic symptoms (low level fever, rash) are observed, but are not usually prevalent and self-antibody development is uncommon (4,5).

The performance of adverse KP effects on the kidney have not been comprehensively studied from the prospective of tissue damages. According to that, the current-focused study was carried out to generate a data profile about expected changes that could be induced in the kidneys of rats due to the use of intraperitoneal KP.

**Materials and Method**

**Animals and experimental design**

The study involved using 24 adult male Wistar rats, weighed at 175-250gm with 10-14 weeks of age, sorted randomly into four groups (six animals per group) that lived in a housing under 22-25°C. One group was treated as a control group, C, which was supplied with distilled water (DW) only. The remaining animals were presented as (50KP), (25KP), or (12.5KP) groups that received 50mg/kg.b.w., 25mg/kg.b.w., or 12.5mg/kg.b.w., respectively, of KP. The experiment was continued for 70 days, and the kidney tissue samples were collected from the scarified animals at the end of day 70 of that experiment.

**Kidney tissue preparation**

Ten percent formalin was used for fixing the tissue specimens for two hours that was followed by a 30-minute-DW based removal step of the fixative. Then, a series of alcohol concentrations at (70% for 30mins, 90% for 60mins, and 100% as two cycles for 60mins per cycle) was used to dehydrate the tissues followed by a 50%:50% of alcohol to xylene immersing step for clearing the tissues for 60mins. After that, the tissues were immersed in a pure xylene for 90mins. Later, molten paraffin wax was used to impregnate the tissues followed by embedding and blocking out those tissues. Paraffin based sections at 4 to 5um were hematoxylin- and eosin-stained. The tissue sections were prepared according to (6–8).

**Results**

The kidney tissues of the 50KP animals revealed dilation of the tubules throughout the outer strip of the outer medulla with necrosis and sloughing of the epithelial cells of the PCTs, figure 1.

![Figure 1: Rat kidney histopathological changes after intraperitoneal ketoprofen treatment at 50mg/kg.b.w. for 70 days. A. Dilation of the tubules throughout the outer strip of the outer medulla. (H&E) Stain.400X. B. Necrosis and sloughing of the epithelial cells of the proximal convoluted tubules. (H&E) Stain.200X.](image-url)
However, the 25KP group suffered lesser grades of epithelial-cell sloughing of the PCTs with lower levels of dilation of tubules than those recorded in the 50KP group, figure 2.

![Image](https://example.com/image1)

**Figure 2:** Rat kidney histopathological changes after intraperitoneal ketoprofen treatment at 25mg/kg.b.w. for 70 days. Changes are shown, here, as lesser grades of epithelial-cell sloughing of the proximal convoluted tubules and lower levels of dilation of tubules than those recorded in the 50KP group. (H&E) Stain.200X.

On the other hand, the kidney tissues of the 12.5KP group showed only dilation in the PCTs, figure 3. The KP group kidney tissues were compared to each other and with the control group, figure 4.

![Image](https://example.com/image2)

**Figure 3:** Rat kidney histopathological changes after intraperitoneal ketoprofen treatment at 12.5mg/kg.b.w. for 70 days. Only dilation in the proximal convoluted tubules is shown. (H&E) Stain.200X.
Discussion

Ketoprofen is a NSAID agent that is used for treating cases mediated by an inflammatory, fever, and/or pain process. The use of the drug as a therapeutic compound has been faced with a wide range of difficulties presented by the appearance of various adverse effects such as peripheral edema and platelet malfunction, drowsiness and headache, skin sensitiveness and photosensitization, and gastric ulcer and bleeding due to cardiovascular, central, dermatological, and gastrointestinal reactions, respectively, (9–13). The adverse KP changes occurred in the kidney have not been fully sorted out. Therefore, the current work was conducted to characterized any histopathological alterations that could happen as responses to the use of KP in three concentrations.

The outcomes of the study unveiled that intraperitoneal ketoprofen treatment at 50mg/kg.b.w. for 70 days demonstrated dilation of the tubules throughout the outer strip of the outer medulla and necrosis and sloughing of the epithelial cells of the PCTs. Ingrasciotta et al., (14) has found that using NSAID drugs such as oxicams, ketorolac, meloxicam, and piroxicam was positively correlated with the increased risk of developing chronic kidney disease (CKD). It has been suggested that utilizing ketorolac may induce CKD with a subclinical property due to acute renal damages (14). This indicates an agreement with current findings that revealed the adverse effects encouraged by the use of the NSAID, KP, in the studied rats. The adverse effects of the KP use in humans can be inferred from a case report of a Turkish woman who received a topical treatment of KP as two times daily for five days who revealed increases in the levels of serum creatinine and urea which suggested an acute renal failure condition in this women (15). The use of KP in pregnant women especially a short time before delivery has been found to increase the risk of renal dysfunction in the neonates (3). The systemic-NSAID based kidney damages can be induced via acute interstitial nephritis due to a dose-independent allergic mechanism with cyclooxygenases 1- and 2-non-selective disruption causing an acute renal failure with reversed functions of the affected tissues (15).

However, those damages in the rat kidneys were correlatively decreased as the KP concentration was reduced. This was completely seen with groups 25KP and 12.5KP that showed lower grades of kidney tissue changes suggesting safer use of the KP with reduced concentrations. It has been recognized that using low doses of KP in children had led to the development of low rates of intense adverse effects with only nausea and vomiting (3).
The present experimental data unveil the side effects generated by the use of the intraperitoneal KP in the examined rat kidneys which should be used as a launching set of information for better use or further study this drug and its side effects in human patients.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding.

References


Association between IL12A Gene of G/A genotype Polymorphism and Pulmonary Tuberculosis Risk in Baghdad Population

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Abstract
The study included eighty samples. This cases-controlled study was performed including fifty Pulmonary tuberculosis patients, their ages ranged from 12 to 77 year (27 female and 23 male) and thirty controls (healthy), their ages ranged from 19 to 58 year (15 female and 15 male). We confined the frequency of IL12A gene (G/A rs568408 genotype) polymorphism by Tetra-ARMS PCR (Tetra amplification refractory mutation system-polymerase chain reaction) technology. Also, we determined the association of IL12A Gene (G/A rs568408 genotype) polymorphism with Pulmonary tuberculosis patients in Baghdad. Statistical results showed significant difference in genotype frequency of IL12A Gene (G/A rs568408 genotype) polymorphism between Pulmonary tuberculosis patients and control (healthy). The G allele shows high frequency in Pulmonary tuberculosis patients comparison with control (healthy) and present related with etiological fraction risk of Pulmonary tuberculosis patients and its ratio 62% in patients and 51.67% in control (healthy), While A allele shows high frequency in control (healthy) comparison with Pulmonary tuberculosis patients and present related with protective fraction of Pulmonary tuberculosis patients and its ratio 48.33% in control and 38% in Pulmonary tuberculosis patients. The genotypes of GG and AA homozygotes shows high frequency in Pulmonary tuberculosis patients comparison with control (healthy), and its ratio 42% and 18% respectively in Pulmonary tuberculosis patients, while its ratio 3.33% and 0% respectively in control (healthy), also GG and AA genotypes appear related with etiological fraction risk of Pulmonary tuberculosis patients, while the GA heterozygote show high frequency in control (healthy) and its ratio 96%, GA genotypes related with preventive fraction of Pulmonary tuberculosis patients. Our findings demonstrate that the IL12A Gene (G/A rs568408 genotype) polymorphism may represent a significant risk factor for pulmonary tuberculosis patients in Baghdad population.

Keywords: IL12A Gene, Pulmonary Tuberculosis, Polymorphisms, Tetra-ARMS PCR

Introduction
The Pulmonary tuberculosis (TB) is a major cause of morbidity and mortality throughout the world, especially in Asia. The statistical data showed 9.6 million new cases and 1.5 million deaths, based on the WHO (World health organization) of 2015 year¹. TB caused by Mycobacterium tuberculosis is an aerobic rode and intracellular pathogenic bacteria which have target the lungs and causative agent of tuberculosis ². Cytokines are pivotal in activation of the cell mediated immunity required for controlling of intracellular growth and eliminating of pathogens ³. Interlukin-12 cytokine play an important role in immune response of Mycobacterium tuberculosis ⁴, and mainly produced by immune cells (macrophages and dendritic cells) ⁵. IL-12 induces T lymphocyte cells and Natural Killer cells to produce pro-inflammatory cytokines such as TNF-α and IFN-γ in the immune response of pulmonary tuberculosis ⁶. IL12A gene is located on chromosome 3 of short arm in region 12 (3p12) ⁷. There is an association of IL-12A gene polymorphism with the risk of pulmonary tuberculosis ⁸. The variability in the IL-12A gene circuit association studies probably confirm of the genetic heterogeneity underlying susceptibility to pulmonary tuberculosis ⁹. The study present association between IL-12 gene and pulmonary tuberculosis risk, by using a panel of single
nucleotide polymorphism providing comprehensive coverage of these genes (10). IL12A gene is hypothesized to be involved in the progression and development of Pulmonary Tuberculosis. Genetic polymorphisms of IL12A gene, was found that genetic variants G/A rs568408 genotype associated with an increased risk of Pulmonary Tuberculosis. The results of this study demonstrate that genetic polymorphism of IL12 pathway may individually or jointly contribute to the sensibility to and prognosis of Pulmonary Tuberculosis TB (3). The study aimed to the finding association between IL12A gene polymorphism in position G/A rs568408 genotype and risk of pulmonary tuberculosis development in Baghdad population.

**Materials and Method**

**Population samples Study**

The population samples Study consisted of 50 patients with pulmonary tuberculosis (27 female and 23 male), there ages range from 12 to 77 years, and 30 control healthy individuals, (15 female and 15 male), and there ages range from 19 to 58 years. All the samples of pulmonary tuberculosis patients were collected from The National Center for Chest and Respiratory Diseases/ Medical City in Baghdad. They had an established diagnosis of pulmonary tuberculosis by the clinical examination and laboratory test.

Genotyping of IL12A gene (G/A rs568408 genotype)

Genomic DNA was extracted by take five ml of blood from each patient and healthy control by venipuncture, later, 2.5 ml was added in to EDTA tubes then DNA was extracted by DNA isolation kit (Promega, USA) and according to manufacture instructions manual. DNA purity was qualified by Nano drop and it was about 1.6 ±1.8. All samples were kept at 20 ºC for further study. Polymorphism of IL12A gene (G/A rs568408 genotype) was examined by using Tetra-ARMS-PCR technology. The PCR reaction was carried out on a DNA template with a pair of specific primers (Alpha DNA, Canada) that designed according to (11). Table (1), 20 μl was the total volume of reaction mix (PioNeer, Korea), and the molecular marker size (Promega, USA) 100-2000 base pair. Tetra-ARMS-PCR programs were summarized in table (2). The genotypes were established by analyzing electrophoresed 2.5% agarose gel stained with diamond dye (Promega).

**Table (1): primer sequences of IL12A gene (G/A rs568408 genotype) by Tetra-ARMS PCR technology**

<table>
<thead>
<tr>
<th>Target Gene</th>
<th>primer</th>
<th>Primer sequences (5’→ 3’)</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL12A gene (G/A rs568408)</td>
<td>Forward outer</td>
<td>5'-AATTTTGGAAATACCATGTAAGTCTGCT-3’</td>
<td>556 bp</td>
</tr>
<tr>
<td></td>
<td>Reverse outer</td>
<td>5'-AGTTAGCTCAGATGCTTCATGATTACC-3’</td>
<td></td>
</tr>
<tr>
<td>IL12A gene (G and A allele)</td>
<td>Forward inner (A allele)</td>
<td>5'-GAAGGATGGGACTATTACATCCACCTA-3’</td>
<td>271 bp</td>
</tr>
<tr>
<td></td>
<td>Reverse inner (G allele)</td>
<td>5'-AAATGTCAAAAATCACTTGATCAGGTCTC-3’</td>
<td>352 bp</td>
</tr>
</tbody>
</table>
Table (2): The cycling condition for Tetra-ARMS PCR program for detection of IL12A gene (G/A rs568408) by outer primer in pulmonary tuberculosis patient and control groups (healthy) samples.

<table>
<thead>
<tr>
<th>Target gene</th>
<th>steps</th>
<th>Temperature (°C)</th>
<th>Number of cycles</th>
<th>Time (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-denaturation</td>
<td>94</td>
<td></td>
<td>300</td>
</tr>
<tr>
<td>IL12A gene (G/A rs568408)</td>
<td>Initial denaturation</td>
<td>94</td>
<td></td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Annealing</td>
<td>65</td>
<td>35</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Extension</td>
<td>72</td>
<td></td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Final Extension</td>
<td>72</td>
<td></td>
<td>5</td>
</tr>
</tbody>
</table>

Statistics

Differences in the frequencies of of IL12A gene (G/A rs568408 genotype) for pulmonary tuberculosis patient in this study with control groups were analyzed with a value P<0.05 by Fisher’s exact test. Odds ratios (OR) and confidence intervals (CI) were calculated using Compare 2 Ver.3.04 software J. H. Abramson (2003-2013). Preventive Fraction (PF) and Etiologic Fraction (EF) results were compared with Hardy-Weinberg equilibrium and according to the software within the following website www.had2know.com.

Results

The genetic polymorphisms of IL12A gene (G/A rs568408) in fifty pulmonary tuberculosis patients with mean age 34.95±1.4 year, and thirty of healthy individuals as a control samples with mean age 26.7±1.9 year. Notably, the two alleles G/A are more present for IL12A gene (G/A rs568408) with GG, GA and AA genotypes in pulmonary tuberculosis group and control (figure 1), use of tetra-ARMS PCR technology in study. The allelic frequency and genotypes distribution for each tested polymorphisms for healthy control and pulmonary tuberculosis patient are presented in table (3). With respect to the IL12A gene (G/A rs568408) polymorphisms, there was a significance in pulmonary tuberculosis patient in compare with control group (P>0.05), and the G and A alleles were different in frequency, so allele G frequency was 62% for pulmonary tuberculosis patient while allele A frequency was 38%, as compared with G and A alleles in control group that it’s frequency was 51.7% and 48.3% (figure 2). The odds ratio (OR) for G allele was 1.53 with confidence intervals (CI) 0.80 to 2.90 at 95 % (Table 3), and it was 0.21 as an etiological fraction (EF), while for allele A there is no significance in pulmonary tuberculosis patient comparison with control and OR was 0.6 with CI 0.34 to 1.25 at 95%, and the value of allele A as preventive fraction (PF) was 0.17 (Table 3). The previous report on polymorphisms of IL12A gene (G/A rs568408) show that may G allele be an etiological fraction and also, it’s describe that the A allele may be a preventive fraction that correlated with the risk of pulmonary tuberculosis patients. The genotyping polymorphisms for IL12A gene (G/A rs568408) by tetra-ARMS PCR technology, there are a genotypes frequency significance in pulmonary tuberculosis patients, so GG and AA genotypes showed the high frequency in pulmonary tuberculosis patients as compared with control (health) group, and it was 42 % and 18 % respectively (Figure 3), also the OR for GG and AA genotypes was 21 and 14 respectively, with CI 2.74 to 161.1 and 0.82 to 238.1 respectively. The GG and AA genotypes presented of association with etiological fraction for risk pulmonary tuberculosis, while for GA genotype the frequency was 40% and 96% for pulmonary tuberculosis patients and control (health) group respectively (Figure3), also the OR was 0.02 and CI was 0.00 to 0.18 and the value for GA genotype as protective fraction was 0.94. Briefly, the result showed that GG and AA genotypes were correlated with the risk of pulmonary tuberculosis, while GA genotype was correlated with the protective fraction of pulmonary tuberculosis in Baghdad Population. The results are
consistent with (8, 10 and 12).

Figure (1): The genotypes of IL12A gene (G/A rs568408) polymorphisms for Pulmonary tuberculosis patient samples. Electrophoretic scheme (M is DNA marker, IL-12 gene 565bp, samples of 1 and 10 GG homozygous (352bp), 2, 4, 6, 8, 11 and 12 AA homozygous (271bp) and 3, 5, 7 and 9 heterozygous).

Figure (2): Allelic frequencies of IL12A gene (G/A rs568408) polymorphisms for Pulmonary tuberculosis patient and healthy samples.

Figure (3): The genotypes frequencies of IL12A gene (G/A rs568408) polymorphisms for Pulmonary tuberculosis patient and healthy samples.
Table (3): The allelic frequency of IL12A gene (G/A rs568408) for Pulmonary tuberculosis patient and healthy samples

<table>
<thead>
<tr>
<th>Target Gene (G/A rs568408)</th>
<th>Allele</th>
<th>pulmonary tuberculosis Patients (%)Number</th>
<th>Control (%)Number</th>
<th>OR (95%CI)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>62 (62%)</td>
<td>31 (51.7%)</td>
<td>1.53(0.8 to 2.9)</td>
<td>0.247</td>
<td></td>
</tr>
<tr>
<td>E.F</td>
<td>38 (38%)</td>
<td>29(48.3%)</td>
<td>0.6(90.34 to 1.25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>38 (38%)</td>
<td>29(48.3%)</td>
<td>0.6(90.34 to 1.25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P.F</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes: OR= Odds ratio, CI= Confidence Interval, P.F= Preventive fraction E.F= Etiological fraction , P<0.05 by Fisher’s test.

Discussion

Interleukine-12 is an immuno-regulatory cytokine, which linked innate and acquired immune responses to mycobacterium through induction of IFN-γ production (13). A series of recent reports in the cytokine pathway suggest that more subtle variants of relevant genes may contribute to susceptibility to tuberculosis at the general population level. To investigate whether polymorphism in the interleukin-12 (IL-12) gene predispose individuals to tuberculosis, we studied these genes by single-strand conformational polymorphism analysis and direct sequencing (6). The most potent enhancer of reactivated T-cells and interferon production which is necessary for killing intracellular bacteria like mycobacteria is interleukin-12 (IL-12), and confirms to be an effective and successful adjuvant to a standard anti-tuberculotic medication in patients suffering from progressive pulmonary tuberculosis (TB) (14). IL-12, produced mainly by macrophages and dendritic cells, has an important role in the immune response to Mycobacterium tuberculosis, also, IL-12 induces T cells and NK cells to produce pro-inflammatory cytokines such as Interferon-γ and Tumor necrosis factor-α while also regulate the production of IL-17 in immunity response for Mycobacterium tuberculosis (6). The genetic polymorphisms of the IL-12 pathway may individually contribute to the susceptibility to and prognosis of pulmonary tuberculosis TB (12). We investigated the impact of IL12A rs568408 gene polymorphisms on risk pulmonary tuberculosis in a sample was living in southeast (13). The IL12A rs568408 variant was not a risk factor for susceptibility to pulmonary tuberculosis in codominant, dominant and recessive tested inheritance models (11), but another study showed association between IL12A rs568408 polymorphisms and risk of pulmonary tuberculosis in Chinese population and It was found that genetic variants AG/GG of rs2243115 (IL12A) were associated with a decreased risk of pulmonary tuberculosis (12).

Conclusion

The statistical data of current study proved the association between of IL12A gene (G/A rs568408) polymorphism and Pulmonary tuberculosis risk in Baghdad Population.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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10. Ayoub Sabri, 1,2,a Audrey V. Grant, 3,4,a Kristel Cosker, 3,4 Safa El Azbaoui, 1,2 Ahmed Abid, 5 Ismail Abderrahmani Rhorfi, 5 Hicham Souhi, 5 Hicham Janah, 5 Kebir Alaoui-Tahiri, 5 Yasser Gharbaoui, 5 Majid Benkirane, 6 Marianna Orlova, 7 Anne Boland, 8 Caroline Desswarte, 3,4 Melanie Migaud, 3,4 Jacinta Bustamante, 3,4,9 Erwin Schurr, 7 Stephanie BoissonDupuis, 3,4,10 Jean-Laurent Casanova, 3,4,10,11 Laurent Abel, 3,4,10,11a and Jamila El Baghdadi1. Association Study of Genes Controlling IL-12-dependent IFN-γ Immunity: STAT4 Alleles Increase Risk of Pulmonary Tuberculosis in Morocco. The Journal of Infectious Diseases 2014; 210:611–8.


The Effect of Allogenic Bone Marrow on Integration Dev-Mesenchymal Stem Cell And Vascular Endothelial Growth Factor

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Abstract

Background: The integration of the tendon graft within the bone tunnel is one of the decisive success factors of the Anterior Cruciate Ligament (ACL) reconstruction. In ACL reconstruction that uses tendons as graft, the healing process that occurs in the bone tunnel is healing with fibrotic tissue.

Aim: This study aims to determine the role of bone marrow mesenchymal stem cells and vascular endothelial growth factor intraarticularly in improving the biomechanical strength.

Methods: This study uses experimental research design by using animal as a testing tool. The animals used in this study are male New Zealand white rabbits (Oryctolagus cuniculus), with the weight ranging of 2000 grams up to 3000 grams.

Results: From the experiment, all samples from both treatment and control groups were all evaluated and no post-operative complication was found. At the evaluation time of 3 weeks, the difference between the mean of maximum strain rate between the treatment group and the control group was not statistically significant (p >0.05). At the evaluation time of 6 weeks, it was found that the difference in mean value of the maximum strain load force between the treatment groups versus the group was statistically significant.

Conclusions: From this study, it can be concluded that intraarticular BM-MSC and VEGF administration can increase the ultimate tension strength of postoperative graft tendon rosary ACL reconstruction.

Keywords: Healing of bone tendon-tunnel, ACL reconstruction, Vascular Endothelial Growth Factor (VEGF), Bone Marrow Derived Mesenchymal Stem Cell (BM-MSC).

Introduction

The process of widening the tunnel is an important concern in ACL reconstruction. This process occurs due to two things, namely the existence of micro-motion and extravasation of synovial fluid into the bone tunnel¹. The ACL post-operative rehabilitation program requires limited mobilization to provide sufficient opportunities for integrated graft within the bone tunnel². One of the critical to the success of ACL reconstruction through the process of integration between tendon graft and bone tunnel³.

Techniques used in ACL reconstruction to improve and improve integration between tendon graft and bone tunnel. The hope is, allowing the sufferer to start and undergo the rehabilitation process and ultimately speed up the chances of the sufferer to immediately return to the activity and return to the sporting activities⁴. Mesenchymal Stem Cell has been assessed to have the potential to improve the quality of integration between tendon graft and bone tunnel.

The ability of stem cells to differentiate and ease in the extraction process is one of the considerations in the use of mesenchymal stem cells in an attempt to enhance the integrity of the tendon graft bone tunnel. Several
studies have mentioned a soft tissue graft augmentation technique using mesenchymal stem cell histologically and biomechanically. Histologically, the tissue formed at the border contact of the tendon graft surface and the bone wall of the bone tunnel resembles the healing of the bone-tendon-bone graft on the reconstruction of the ACL. Another study mentions a histological picture of integration that resembles chondral enthesis of native ACL insertion.

In addition, it is biomechanically mentioned that the augmented graft tendon with mesenchymal stem cell has a failure limit on higher loads when compared with other augmentation methods. This is due to the widening of the tunnel. One theory has explained the influence of synovial fluid within the joints, in which the synovial fluid has the effect of inhibiting the process of forming the hematoma.

Bone marrow derived mesenchymal stem cells (BMSCs) are a new source of cells to help speed the healing and regeneration of new tissues. BMSCs are able to differentiate into osteoblasts that will form cartilages to form fibrocartilage tissue. Healing with fibrocartilage resembles healing in the bone-tendon-bone graft type. Biomechanical studies that have been done also provide results of fibrocartilage healing on bone-tendon-bone graft better than the healing fibrocollagen on soft tissue graft. Furthermore, histologically, fibrocartilage healing may provide an image resembling chondral enthesis in normal native ACL insertions.

One of the problems with the ACL reconstruction process is the occurrence of hypoxia in the automotive tendon tissue after harvesting or harvesting. A study in Japan has proven that VEGF can improve vascularization and improve the quality of fibroblast tissue integration. In other side, VEGF was evaluated based expression identified through immunohistochemical staining. Based on the description above, this study aims to determine the effect of intra articular bone marrow derived stem cells (BMSCs) and vascular endothelial growth factor (VEGF), to increase the biomechanical strength of the autograft tendon integrity in the bone tunnel in retaining the pull-out force ACL reconstructions.

Method

This study uses experimental research design by using animal as a testing tool. The animals used in this study are male New Zealand white rabbits (Oryctolagus cuniculus), with the weight ranging of 2000 grams up to 3000 grams. The study was conducted from June to August 2014 for the research procedure, while it takes one month (August 2014-September 2014) for the analysis of the results and the preparation of the research report. This study located at Tropical Disease Center (TDC) Airlangga University.

The anesthesia drugs used were a combination of xylazin with a concentration of 100mg/cc and ketamine, a minor surgical base set, consisting of a scalpel, tweezers, tissue scissors, skin retractor, scissors of yarn, and pean clamp, sewing threads consisting of ethibond 4.0 for graft fixation and monofilament absorbable syntetic measures 4.0 to cover the capsule and field operation. The research instrument used a ruler to measure elongation and translational distance (drawer), Autograph AG-10E engine (Shimadzu Corporation, Japan) to measure the forces required to create pullouts or ruptures from tendon graft.

After doing the research result of evaluation and biomechanical test collected then done analysis and statistical test. Data collected from result of evaluation from second time done recapitulation, tabulation, and statistical analysis. All data were analyzed using IBM SPSS v.21.0 for windows program (SPSS. Inc. Chicago, IL).

Results

Based on the results of data analysis obtained the results of posterior anterior translational distance measurement at various angles (Table 1).
Table 1. Measurements of posterior anterior translation at various angles and ultimate tension strength measurements and types of damage occurring in the tendon graft during evaluation periods 3 and 6 weeks.

<table>
<thead>
<tr>
<th>Group</th>
<th>Unit Exp</th>
<th>Anterior-Posterior Translation (mm)</th>
<th>Ultimate Tension Strength</th>
<th>Type of Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30°</td>
<td>60°</td>
<td>90°</td>
</tr>
<tr>
<td>3-week control</td>
<td>K3.1</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>K3.2</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>K3.3</td>
<td>3</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>K3.4</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>K3.5</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>K3.6</td>
<td>2</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>6-week control</td>
<td>K6.1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>K6.2</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>K6.3</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>K6.4</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>K6.5</td>
<td>2</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>K6.6</td>
<td>1</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>3-week treatment</td>
<td>P3.1</td>
<td>4</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>P3.2</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>P3.3</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>P3.4</td>
<td>1</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>P3.5</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>P3.6</td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>6-week treatment</td>
<td>P1.1</td>
<td>2</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>P1.2</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>P1.3</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>P1.4</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>P1.5</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>P1.6</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

From the table 1, after the calculation of mean and standard deviation of anterior-posterior translation distance that occurred in each group both control and treatment group (Table 2). Here is result for table 2.
Table 2. The Mean Result of Posterior Anterior Translational Distance at Various Angles and Ultimate Tension Strength.

<table>
<thead>
<tr>
<th>Variable of anterior-posterior translation gap</th>
<th>Group</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treatment</td>
<td></td>
</tr>
<tr>
<td>Duration</td>
<td>3 weeks</td>
<td>6 weeks</td>
<td>3 weeks</td>
</tr>
<tr>
<td>Translation Angle</td>
<td>30°</td>
<td>2.50±0.837</td>
<td>2.33±1.033</td>
</tr>
<tr>
<td></td>
<td>60°</td>
<td>3.00±0.894</td>
<td>3.83±1.169</td>
</tr>
<tr>
<td></td>
<td>90°</td>
<td>3.50±1.378</td>
<td>4.00±1.549</td>
</tr>
<tr>
<td>Variable of ultimate tension strength</td>
<td>Groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Treatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 week</td>
<td>6 week</td>
<td>3 week</td>
</tr>
<tr>
<td></td>
<td>0.0197±0.010</td>
<td>0.046±0.012</td>
<td>0.018±0.007</td>
</tr>
</tbody>
</table>

From that result, statistical analysis in both groups was based on two observation times (3 and 6 weeks). From the two groups, a comparative test was performed using Mann-Whitney test method. Normality test results using Shapiro-Wilk test showed the results p >0.05 indicating the data is not normally distributed. Therefore, the test used was Mann-Whitney test (Table 3).

Table 3. Data distribution normality test results for the mean of anterior-posterior translation at various angles and Shapiro-Wilk test results.

<table>
<thead>
<tr>
<th>Normality Test</th>
<th>Group</th>
<th>Kolmogorov-Smirnov*</th>
<th>Shapiro-Wilk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Statistic</td>
<td>Sig.</td>
<td>Statistic</td>
</tr>
<tr>
<td>Translation 300</td>
<td>3 week control</td>
<td>0.392</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>6 week control</td>
<td>0.293</td>
<td>0.117</td>
</tr>
<tr>
<td></td>
<td>3 week control</td>
<td>0.277</td>
<td>0.168</td>
</tr>
<tr>
<td></td>
<td>6 week treatment</td>
<td>0.492</td>
<td>0.000</td>
</tr>
<tr>
<td>Translation 600</td>
<td>3 week control</td>
<td>0.202</td>
<td>0.200*</td>
</tr>
<tr>
<td></td>
<td>6 week treatment</td>
<td>0.277</td>
<td>0.168</td>
</tr>
<tr>
<td></td>
<td>3 week treatment</td>
<td>0.254</td>
<td>0.200*</td>
</tr>
<tr>
<td></td>
<td>6 week treatment</td>
<td>0.202</td>
<td>0.200*</td>
</tr>
</tbody>
</table>
Table 3. Data distribution normality test results for the mean of anterior-posterior translation at various angles and Shapiro-Wilk test results.

<table>
<thead>
<tr>
<th>Test</th>
<th>3 week-evaluation</th>
<th>6 week-evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Translation 30°</td>
<td>Translation 60°</td>
</tr>
<tr>
<td></td>
<td>Translation 30°</td>
<td>Translation 60°</td>
</tr>
<tr>
<td>Mann-Whitney Test</td>
<td>0.21</td>
<td>0.867</td>
</tr>
<tr>
<td>Shapiro-Wilk Test</td>
<td>0.849</td>
<td>0.235</td>
</tr>
</tbody>
</table>

Evaluation of 3 weeks p >0.05

Evaluation of 6 weeks at 30° dan 60° p >0.05 and translation 90° p < 0.05

From table 4 there was a significant difference of AP translation distance at 90 degree knee angle between control and treatment group (significancy 0.020, p <0.05) (Table 4). The control group at 3 weeks evaluation period had a mean value of 0.0197, the control group with the evaluation period of 6 weeks had a mean value of 0.046 while the treatment group with 3 weeks evaluation had a mean value of 0.018 and in the treatment group with 6 weeks evaluation period had a mean value of 0.0698 (Table 2).

Shapiro-Wilk test results obtained significance value 0.291, 0.150, 0.058 and 0.337. Because the value of p >0.05 it can be concluded that the data distribution for each group is normal, so for comparative test that can be done is by t-test unpaired (independent student t test).

For comparison group comparison and treatment at 3 weeks evaluation, the result of significancy was 0.801. Because p >0.05 it can be concluded that there was no significant difference between pullout strength / ultimate tension strength of control group and treatment during evaluation period 3 weeks.
Table 5. The result of independent test of T test comparing the ultimate value of tension strength between the two groups during the evaluation period of 3 weeks and 6 weeks

<table>
<thead>
<tr>
<th>Variable</th>
<th>3 weeks</th>
<th>6 weeks</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (N=6)</td>
<td>Treatment</td>
<td>T-test</td>
</tr>
<tr>
<td>Pull-out strength</td>
<td>0.1967±0.10328</td>
<td>0.01833±0.07202</td>
<td>0.801</td>
</tr>
</tbody>
</table>

The biomechanical test was performed in 3 weeks postoperative control group and 6 tendon graft was ruptured on the intraarticular (midsubstance) part (100%) while the 3-week treatment group also obtained 4 graft tendon rupture on the intra-articular (midsubstance) (66.7%) and 2 graft tendons had pullout on the bone tunnel of the femur (33.3%).

Discussion

ACL reconstruction often uses a hamstring tendon as one of the tendon graft options. Many studies have studied aspects of the use of hamstring tendons as grafts, ranging from techniques, biomechanical, histologic aspects as well as the results of this procedure.

The success of the ACL reconstruction procedure using a hamstring tendon as a tendon graft depends not only on the initial fixation when the graft mounting operation is performed, but on the biological integration of the longitudinal graft in the bone tunnel that determines long-term outcomes, but the factors affecting the biological integration of the tendon graft on the bone tunnel remains unclear. However, from a variety of materials used, there has been no research that proves the superiority of a material that significantly outweighs other additions.

Biomechanical testing is one of the most commonly used methods to study the effectiveness of a material used as an augmentation to accelerate or increase the integration strength of tendon graft in a bone tunnel. There are different histologic features that occur in the mouth of the bone tunnel or the entrance and along the bone tunnel. In the study also mentioned that the position of the tendon graft insertion in the bone tunnel also has a role to play in the resulting force and work on the tendon graft. This is important given the various techniques and tunnel positions used in actual human ACL reconstruction.

One of the things that happen to the post-reconstruction tendon graft is the occurrence of reduction in tendon stiffness. This results from the effects of VEGF on the tendon graft which results in a decrease in the stiffness of the tendon graft itself. In the present study this phenomenon can be observed by the posterior anterior-posterior translation of the postoperative femur-graft-tibia complex compared between the control and treatment groups.

The results of this study differ from those shown by previous studies that mention significant differences in mean AP translation distances between control groups and treated groups in the form of VEGF. But at the beginning of postoperative graft tendon histologic features obtained in the form of new vascular formation areas, infiltration of new cells and the formation of new collagen fibers that have not been arranged regularly. The formation of new vascular tissues, irregular infiltration of cells and collagen fibers is a “weakness” of the tendon graft structure used. In addition external VEGF applications also enzyme matrix metalloproteinase (MMP) by several types of cells. The result of mean comparison of AP translation distance between control and treatment group in this study showed that there was no significant difference between the two groups except in the treatment group at 90 degree angle at 6 weeks evaluation.
This is consistent with the results of previous studies showing no significant difference between ultimate tension strength between the tendon groups receiving VEGF and the control group\(^7\). However, in this study for comparisons of 6 weeks evaluation results showed significant differences. In the control group, the tendon graft underwent ischemic and transient necrosis following harvesting, but after implantation, there was a normal healing process through fibroblast tissue\(^3\).

**Conclusion**

From this research it can be concluded if there is no significant difference from AP translation distance that reflects the stiffness of the tendon graft between reconstruction of ordinary ACL and reconstruction ACL coupled with intraarticular BM-MSC and VEGF delivery.

**Conflict of Interest**: Nil

**Source of Funding**: Self

**Ethical Clearance**: This study was approved by Ethical Commission of Health Research Faculty of Medicine University of Airlangga.

**References**


7. Фоменко СМ, Прохоренко ВМ, Симагаев РО, Киселев АС. ОПЫТ ХИРУРГИЧЕСКОГО ЛЕЧЕНИЯ ПЕРЕДНЕЙ НЕСТАБИЛЬНОСТИ КОЛЕННОГО СУСТАВА С ПРИМЕНЕНИЕМ ДВОЙНОЙ ФЕМОРАЛЬНОЙ ФИКСАЦИИ АУТОТРАНСПЛАНТАТА ПЕРЕДНЕЙ КРЕСТООБРАЗНЫЙ СВЯЗКИ.


Influence of BRACA Usage on Self Improvement of Breast Cancer Patient by Post Mastectomy

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Abstract

Background: Breast cancer is an abnormal tissue, rapid growth and uncontrolled cells found in breast tissue. This body image disorder is thought to be related to the occurrence of post-mastectomy depression.

Objectives: To know the influence between the use of BRACA with self-image improvement in patients with post-mastectomy breast cancer in Dr. Soetomo General Hospital, Surabaya.

Method: The subject was a patient who has undergone a mastectomy on one side of the breast. The study design was a queasy experimental type of pre-post control design by sorting the sample and dividing it into 2 groups where 1 control group (not using BRACA) and another group in intervention using BRACA. Statistical analysis using t-test.

Result: There was an increase in the self-image of respondents who intervened with BRACA’s use of pre and post use by showing a significant effect between BRACA use and self-improvement of post-mastectomy breast cancer subjects in Dr. Soetomo General Hospital, Surabaya (p = 0.000).

Conclusion: The major cases occur on the right side of the breast with the duration of surgery more than one month. BRACA can be used as an alternative to improve the patient’s self image after mastectomy as it increases the self-image of the patients.

Keywords: BRACA, Self-image, Breast Cancer

Introduction

In Indonesia, breast cancer known as the most common cancer experienced by women that can lead to death ¹,², and can be evaluated through common sign such as menstrual disorders ³. Breast cancer has a physical and psychological impact on the individual. Physically there is a change due to the disease process and treatment. Changes that occur due to disease processes include changes in shape, size, or texture of the breast due to an enlarged tumor mass. Changes that can occur in the form of partial or total loss of the breast and the scar due to surgery ⁴.

Chemotherapy, Intraoperative radiotherapy, and oncoplastic are the techniques to treat early stage breast cancer ⁵,⁶. For further stage, partial or total loss of the breast or the effects of chemotherapy may result in negative psycho-social consequences including one of the problems in the woman’s self-image ⁷,⁸. The study of 112 breast cancer patients showed 33.00% of women felt different from others after surgery, 12.00% felt others were aware they were undergoing treatment and made 25.00% of them worried and 50.00% of patients were disturbed by their body changes after surgery⁹. The distortion of self-image in the mastectomy patient is worse than that of the amputated patient ¹⁰. Research on patients with mastectomy that acts to cover up the loss of self-image by wearing a wide headscarf, wearing a long jacket or wearing a loose shirt but the respondent declared reluctant to use a bra for reasons the wound is still not dry, uncomfortable or feeling asymmetrical on one of his limbs.
The literature study of 45 research articles on breast cancer patient’s patient is proposed by various coping strategies used by women who have breast cancer to face both physical and psychological challenges. Some types of coping strategies used are effective/adaptive coping (such as active problem solving, and acceptance of disease/diagnosis) to maladaptive coping (such as self-blame and denial). Psychological distress such as poor self-image becomes a predisposing factor to chronic depression, eating disorders, use of illicit substances and other attitude. Several studies have found an association between coping with the self-image of post-mastectomy patients. Based on the above phenomenon researchers interested in researching and creating a tool that we named “BRACA” which can reduce the self-image disturbance experienced by patients with breast cancer primarily post-mastectomy patients or after removal of breast.

**Methods**

The subjects were breast cancer patients who had performed radical mastectomy action in Dr. Soetomo General Hospital, Surabaya. The inclusion criteria were post-mastectomy patients either breasts (either right or left), age between 30-60 years old, minimum primary school (SD) to Bachelor (S1) education, the patient was confirmed for control again within a minimum of 1 week.

The study design used quasy-experimental type pre post control design by sorting samples on entry into the room, dividing it into 2 groups where 1 group as control (not using “BRACA”) and other group intervened using “BRACA”. The sampling technique used purpuseful sampling. The location of this research was conducted in Dr.Soetomo General Hospital, Surabaya in March to May 2017. This research has obtained the approval of ethical clearance from the ethics commit- tee of Dr. Soetomo Teaching Hospital, Surabaya In- donesia.

Subjects will be retrieved data of anamnesis in the form of demographic data such as age, type of work, education, marital status, religion, and insurance in health financing, while the collection of clinical data can be in the side of the mastectomy breast, postoperative length, and use of braces or not. BRACA or Bodyfit Range Cancer is a tool created by researchers based on cotton that can absorb sweat. Inside the BRACA there are 2 versatile pockets that can be filled with foam pads or can also be filled with jelly that can be used as a cold compress (Figure 1). Measurement of self-image on the subject can be done with self-image questionnaire as many as 15 questions with yes or no answer. Statistical analysis used the t-test with significance value $\alpha = 0.05$ with SPSS version 11.5 for Windows (SPSS, Inc., Chicago, IL). While to know the degree of the power relationship between variables measured by the correlation coefficient ($r$).

**Result**

Demographic and Clinical Data Subject Research

The majority of subjects aged was 35-50 years (53.20%), worked as private/entrepreneurs (61.70%), junior high school (76.61%), married (83.00%), Moslem (87.00%), followed more than 1 social (63.80%) and financing by using National Health Organization (BPJS) (83.00%). The majority of subjects in the right side of the breast mastectomy (80.90%) and duration of surgery for more than one month (100.00%).

**Correlation Use of BRACA Pre and Post-Test with Self Image**

The pre-test showed no subjects with high self-image in the treatment group by using BRACA and in the control group was obtained more than a half had high self-image (64.00%). In the post-test, there were no subjects with a low self-image in the treatment group using BRACA and in the control group, all had high self-image (100.00%). So there was a strong positive relationship between BRACA usage with self-image enhancement ($p = 0.000$).

**Table 1. Correlation of BRACA Pre and Post-Test on Self Image**

<table>
<thead>
<tr>
<th>Self-image</th>
<th>Pre-Test With BRACA</th>
<th>Post-Test With BRACA</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>0.00</td>
<td>86.00</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>64.00</td>
<td>25.00</td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>60.00</td>
<td>14.00</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>40.00</td>
<td>0.00</td>
<td></td>
</tr>
</tbody>
</table>

BRACA=Bodyfit Range Cancer
Discussion

The result of the statistical test shows that there is a strong positive relationship between BRACA usage with self-image improvement in post-mastectomy breast cancer patient. In the result of the post-test questionnaire also showed that there were no subjects with a low self-image in the treatment group using BRACA whereas previously in pre-test was obtained 40.00% subjects had a low self-image.

Women who suffer from breast cancer usually experience physical and psychological changes that can affect the appearance and activity of women in their daily life, because of cancer associated with physical problems of pain, misery, death and moderate cost of psychosocial problems that are anxious, body image and loss. Body image changes almost in all patients with breast cancer, especially in patients who do the mastectomy. If these changes are not integrated with a healthy self-concept then the quality of life will be decreased drastically even will threaten the patient’s safety. In general, the factors that affect women have body image disorder according to the body image distortion and body image dissatisfaction. Some factors may affect one’s self-concept including personal factors that include: age, sex, education, occupation, economic status, lifestyle, and personality.

Then, of the 22 people included in the treatment group, 3 respondents still had an elementary education. Education also affects the self-concept and emotional maturity of a person. A person with higher education will better understand and understand about the disease he suffered. The higher a person’s education is expected to have better self-concept and high self-image.

From the characteristics of marital status was obtained data by 20 people in the treatment group with BRACA is has a married status and only 2 people are widow status. Family support (especially the role of husband) is very influential on the condition of one’s self-image. Use of BRACA in patients can improve the self-image of the patient, especially in front of her husband. The use of BRACA makes the breasts will look prominent and symmetrical so that patients will feel more confident in front of her husband.

The majority of 61.70% subjects had a private job type, the occupation may affect patient satisfaction with health services, because the working patient is a productive patient, usually more sensitive to the circumstances around, with the patient’s activity more likely to interact with the surrounding environment. When associated with pre-test and post-test results that increase can be explained as follows that with the use of BRACA then the breasts will look like a normal woman so that other patients will become more confident and self-actualization will be increased.

Conclusion

The majority of subjects in the mastectomy of the right side of the breast and the duration of surgery more than one month. There is an increase in self-image after BRACA use in patients who have been in mastectomy. Support is needed in the form of post-operative guidance counseling and involvement of support groups and families in order not to experience post-mastectomy depression.

Ethical clearance

This research involves participants in the process using a questionnaire that was accordant with the ethical research principle based on the regulation of research ethic regulation. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, non-maleficence, and justice.

Conflict of Interest: The authors have not found any conflict of interest related to this research so far.

Source of Funding: All of the cost and fees related with this research are paid by the authors only with no sponsorship nor external funds.

References

Correlation of Quadriceps and Hamstring Muscles Strength with Knee Osteoarthritis Stages and Pain Levels in Elderly Female Subjects

Mohammad Fathul Qorib, Hening Laswati

Abstract

Background: Osteoarthritis (OA) is one of the most common disease occur in elderly. It is commonly occurs in knee, hips and interphalangeal joints. Pain caused by knee OA may result in functional disorders that affect patient’s life quality.

Objective: To analyze correlation of quadriceps and hamstring muscles strength with knee OA stages and pain levels in elderly female subjects.

Methods: 38 elderly female subjects (average age of 67.18 ± 4.91) were assessed for pain scale using numeric rating scale (NRS). We used 1RM protocol (EN-Tree) to measure quadriceps and hamstring muscles strength. We performed anterior-posterior and lateral knee X-rays to determine OA stage. The process of data analysis was conducted using several tests, including Kolmogorov-Smirnov test, Pearson’s correlation test, Spearman’s correlation test and Mann-Whitney test to measure difference between knee OA level using 1RM quadriceps muscle and quadriceps/hamstring (Q/H) ratio.

Results: The average NRS was 3.68 ± 1.19, 1RM quadriceps and hamstring muscles strength were 2.68 ± 1.23 and 1.11 ± 0.44, respectively, quadricep/hamstring ratio (Q/H) was 2.53 ± 0.95. A total of 30 subjects had OA stage 2, while 8 subjects had OA stage 3.

Conclusion: The 1RM quadriceps and hamstring muscles was not correlated with NRS. There was no difference in 1RM quadriceps and hamstring muscles between subjects with OA stage 2 and 3. We found a positive correlation between Q/H ratio and the NRS. The Q/H ratio in subjects with OA level 2 and 3 was different.

Keywords: 1RM Quadriceps and Hamstring, NRS, osteoarthritis level, Q/H ratio

Introduction

Osteoarthritis (OA) is the most common joint disorder and a major cause of disability in elderly people. Pain caused by knee OA may result in functional disorders that affect patient’s life quality. Prevalence increases with age. Ten percent of total population will have symptomatic osteoarthritis in their 70s. The number and percentage of elderly in Indonesia continues to increase each year with an average increase of 0.5% per year. Such increases will potentially lead to an increased OA prevalence. Osteoarthritis may cause socio-economic problems in family and social environment.

Osteoarthritis is a common progressive joint disorder. It is commonly occurs in knee, hips and interphalangeal joints. Even though OA is not commonly found in
knee joint, it is the most common cause of disability. Patients with knee osteoarthritis often experience disruption in their daily activities because of the arising pain. Female subjects have a greater risk of knee OA than male. The number of new patients with knee OA visiting Medical Rehabilitation Unit of Dr. Soetomo Teaching Hospital, Surabaya, Indonesia, from October 11- December 28, 2011 were 61 patients (51 females and 10 males). The osteoarthritis pathology is damages in joint cartilage and new subchondral bone formation (osteophyte). Osteoarthritis occurs due to disruption of the balance between cartilage catabolism and anabolism. Some factors affecting the balance are types of work, weight, trauma, abnormal growth process, collagen gene mutations, muscle weakness, changes in proprioceptive function, joint neurological disorders and metabolic abnormalities.

Muscle strength decreases with age particularly after 60 years old. Muscle strength will be reduced by 45% at age 65 due to a complex process including biological and functional causes. Quadriceps and hamstring muscles are the most common muscle types suffering from strength and size reduction. Muscles around the knee act as chondro-protective. A study conducted in 178 female and 164 male elderly found that quadriceps muscle weakness became a risk factor for knee OA occurrence in the female patients.

Other studies in elderly subjects found that quadriceps muscle weakness was not the result of knee immobilization due to pain, but rather a trigger factor of knee OA. Quadriceps muscle weakness eliminates one of knee stabilizer functions. Several studies have shown that quadriceps receptor failure when performing voluntary contractions is one of the causes of decreased muscle strength occurring in old age.

Hamstring muscles have a role as knee joint stabilizer. To date, there has been no study confirming correlation of quadriceps and hamstring muscle strength with pain level and knee OA, therefore is still no form of early prevention and a more measurable muscle strengthening program for pain management due to knee OA. Several previous studies have examined the relationship between quadriceps muscle weakness and OA. Those studied did not radiologically examine correlation of hamstring muscle strength with pain OA levels radiologically.

Muscle strength assessment can be easily conducted with simple and inexpensive methods. One method used to assess muscle strength is 1RM (repetition maximum), a maximum load that can be lifted with the movement of the full joint motion, or calculated according to the Oddvar Holten diagram. Previous studies conducted in healthy women subjects found no significant difference of quadriceps bench after being assessed with 1RM using EN-tree tools. Disability in elderly subjects due to knee OA pain complaint should be prevented by an early 1RM assessment and an immediate reinforcement program if it is proven that quadriceps and hamstring muscle strength is correlated with pain level and knee OA. Quadriceps bench can be made by yourself or modified easily and at very cheap cost. Muscle strength is easily assessed and enhanced by exercise using quadriceps bench. In this study, we used the EN-tree tool because the process of collecting 1RM data of quadriceps and hamstring muscles was more objective and more efficient. This study aims to analyze correlation of quadriceps and hamstring muscles strength with knee osteoarthritis (OA) stages and pain levels in elderly female subjects.

**Methods**

This observational analytic study using cross sectional approach was carried out from March to April 2012 at Medical Rehabilitation Unit of Dr. Soetomo Teaching Hospital, Surabaya, Indonesia. We used 38 elderly female subjects with knee OA who visited Medical Rehabilitation Unit of Dr. Soetomo Teaching Hospital, Surabaya, Indonesia. The inclusion criteria were as follows: (1) Women aged 60 years and over; (2) Willing to participate in this study by signing informed consent. On the other hand, the exclusion criteria were: (1) Obese subject with a Body Mass Index (BMI) of more than 30; (2) Having experienced lower extremity fracture; (3) Having impaired neurological function and cardiorespiratory disorders. The study protocol was approved by the ethics committees of Dr. Soetomo Teaching Hospital (Surabaya, Indonesia).

This study used EN-Tree protocol with NRS to assess pain level at Medical Rehabilitation Unit and X-ray machine at Radiology Unit of Dr. Soetomo Teaching Hospital, Surabaya, Indonesia. We assessed if subjects had not experienced any lower extremity fractures and/or neurological disorder. Pain level was assessed using NRS, while 1RM was calculated using EN-Tree protocol. Knee was evaluated using X-ray.
All data were processed using SPSS software (SPSS., Inc., Chicago., IL). The process of data analysis was conducted using several tests, including Kolmogorov-Smirnov test, Pearson’s correlation test (for normal data distribution), Spearman’s correlation test (for non-normal data distribution) and Mann-Whitney test to measure difference between knee OA level using 1RM quadriceps muscle and quadriceps/hamstring (Q/H) ratio.

Results

We took 1RM quadriceps and hamstring muscle data from 38 eligible subjects using EN-Tree protocol at Medical Rehabilitation Unit of Dr. Soetomo Teaching Hospital, Surabaya, Indonesia. Radiographic data of knee OA was taken after 1RM examination. All subjects had bilateral knee OA, but we only assessed the right side. The X-ray results showed 30 subjects had stage 2 OA, and 8 subjects had stage 3 OA. The subjects’ basic characteristics were presented in Table 1. The data for age, BMI, NRS and 1RM hamstring muscle parameters were normally distributed, while data for 1RM quadriceps muscle and Q/H ratio were non-normally distributed. Pearson’s correlation test showed no significant correlation between pain level with age, BMI and 1RM hamstring muscles.

Table 1. Subject’s Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Mean ± SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>38</td>
<td>67.18 ± 4.91</td>
<td>62.27</td>
<td>72.09</td>
</tr>
<tr>
<td>BMI (Kg/m2)</td>
<td>38</td>
<td>24.47 ± 3.09</td>
<td>21.38</td>
<td>27.56</td>
</tr>
<tr>
<td>NRS</td>
<td>38</td>
<td>3.68 ± 1.19</td>
<td>2.49</td>
<td>4.87</td>
</tr>
<tr>
<td>1RM Quadriceps (Kg)</td>
<td>38</td>
<td>2.68 ± 1.23</td>
<td>1.45</td>
<td>3.91</td>
</tr>
<tr>
<td>1RM Hamstring (Kg)</td>
<td>38</td>
<td>1.11 ± 0.44</td>
<td>0.67</td>
<td>1.55</td>
</tr>
<tr>
<td>Q/H Ratio (Kg)</td>
<td>38</td>
<td>2.53 ± 0.95</td>
<td>1.58</td>
<td>3.48</td>
</tr>
<tr>
<td>OA Stage</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In Table 2. Shown the data for normality test for variables.

Table 2. Normality Test for Age, BMI, NRS, 1RM Quadriceps Muscle, 1RM Hamstring Muscle and Q/H Ratio Parameters

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>38</td>
<td>0.465</td>
</tr>
<tr>
<td>BMI (Kg/m2)</td>
<td>38</td>
<td>0.934</td>
</tr>
<tr>
<td>NRS</td>
<td>38</td>
<td>0.150</td>
</tr>
<tr>
<td>1RM Quadriceps (Kg)</td>
<td>38</td>
<td>0.029</td>
</tr>
<tr>
<td>1RM Hamstring (Kg)</td>
<td>38</td>
<td>0.101</td>
</tr>
<tr>
<td>Q/H Ratio (Kg)</td>
<td>38</td>
<td>0.046</td>
</tr>
</tbody>
</table>
Spearman’s rho showed a weak negative correlation between 1RM quadriceps muscle and pain level. The Q/H ratio had a weak positive correlation with pain level ($r = 0.365$).

**Table 3. Correlation between 1RM Hamstring Muscle, 1RM Quadriceps Muscle and Q/H Ratio with Pain Level**

<table>
<thead>
<tr>
<th>Variable</th>
<th>NRS</th>
</tr>
</thead>
</table>
| Age            | $r = -0.076$
|                | $p = 0.642$
| BMI            | $r = -0.198$
|                | $p = 0.234$
| 1RM Hamstring  | $r = -0.297$
|                | $p = 0.070$
| 1RM Quadriceps | $r = -0.960$
|                | $p = 0.567$
| Q/H Ratio      | $r = 0.365$
|                | $p = 0.024$

**Table 4. Differential Test of Age, BMI and 1RM Hamstring in Both Groups**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Stage OA</th>
<th>N</th>
<th>Mean±SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>2</td>
<td>30</td>
<td>67.033±4.91</td>
<td>0.719</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>8</td>
<td>67.75±5.20</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>2</td>
<td>30</td>
<td>23.97±3.22</td>
<td>0.056</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>8</td>
<td>26.31±1.69</td>
<td></td>
</tr>
<tr>
<td>1RM Hamstring</td>
<td>2</td>
<td>30</td>
<td>1.073±0.45</td>
<td>0.352</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>8</td>
<td>1.24±0.38</td>
<td></td>
</tr>
</tbody>
</table>

T-test showed that age, BMI and hamstring muscle strength between subjects with stage 2 and 3 knee OA were not different, with $p >0.05$ (Table 4.).

**Table 5. Differential Test of 1RM Quadriceps and Q/H Ratio in Both Groups**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Stage OA</th>
<th>N</th>
<th>Mean</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1RM Quadriceps</td>
<td>2</td>
<td>30</td>
<td>2.7733</td>
<td>2.7000</td>
<td>0.60</td>
<td>6.20</td>
<td>0.425</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>8</td>
<td>2.3375</td>
<td>2.7000</td>
<td>1.20</td>
<td>2.70</td>
<td></td>
</tr>
<tr>
<td>Q/H Ratio</td>
<td>2</td>
<td>30</td>
<td>2.6821</td>
<td>2.2404</td>
<td>1.00</td>
<td>4.43</td>
<td>0.047</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>8</td>
<td>1.9692</td>
<td>2.0385</td>
<td>1.20</td>
<td>2.70</td>
<td></td>
</tr>
</tbody>
</table>
On the other hand, Mann-Whitney test showed that the Q/H ration between two groups was significantly different, with \( p = 0.047 \). The average value of the Q/H ratio in the group with stage 3 knee OA was 1.969 (Table 5).

**Discussion**

Pain is an uncomfortable sensory and emotional experience associated with existing tissue damage or potentially causing tissue damage. Uncomfortable feelings include all emotional processes such as fear, sadness, anxiety, or even suicidal attempts. Pain assessment is influenced by various factors including sensory, motoric, emotional and even cultural factors. In this study, we found that subject’s pain was positively correlated and statistically significant with the Q/H ration. This result indicated that the greater the Q/H ratio, the greater the pain level. Increased Q/H ratio in this study correlated with the increase of 1RM quadriceps muscle value and the decrease in value of 1RM hamstring muscle. These findings were consistent the results of previous studies, where the value of Q/H ratio in OA subject was greater than healthy subject, even though it was not significantly different. On the other hand, some studies found the opposite result that the Q/H ration in OA subject was lower than healthy subject \( 13,14 \). Differences in the results of Q/H ratio in some of studies might be due to differences in strength assessment procedures, instruction given to subjects, subject’s position during examination (prone, supine, or sitting), subject’s stabilization during examination \( 15 \). 1RM quadriceps and hamstring muscles negatively correlated with pain level, even though it was statistically insignificant with a weak correlation coefficient (<0.5).

This negative correlation might be due to knee joint stability towards mechanical stress ismore determined by the balance between quadriceps and hamstring muscle strength, suggesting that the Q/H ratio in healthy subject is 3:2 \( 13,14 \). Another assessment of thigh muscle strength ratio is the ratio of concentric strength of hamstring-quadriceps muscles (Hcon/Qcon). The reported Hcon/Qcon ratio value is 0.43-0.90. Previous study suggested that knee injuries could be predicted by detecting the strength balance of both muscles by assessing the Hcon / Qcon ratio of at least 0.6 \( 16 \). The athlete’s and tennis player’s normal H/Q ratio is 60% to 80%. This indicates that the increased strength of quadriceps muscle that is not compensated with the increased strength of hamstring muscle of at least 60% makes the knee will be prone to injury. Quadriceps and hamstring muscles play an important role as structural stabilizers of patellofemoral joints. Quadriceps muscle strength that is not compensated with hamstring muscle strength will cause an excessive tibia translation to anterior and an excessive attraction of the ligaments around the knee joint, particularly ACL \( 17 \).

We found no difference in quadriceps and hamstring muscle strength between group of subjects with stage 2 and 3 knee OA (\( p = 0.352 \)). This might be due to the knee joint stability towards mechanical stress is more determined by the balance between two muscle strength. These imbalances lead to changes in knee joint stability, exaggerated anterior tibial translation and changes in path patellar movement (Patellar tracking), that subsequently result in increased mechanical stress. Excessive mechanical loads and mechanical stresses cause various changes in chondrocytes environment that subsequently result in decreased tissue pH and lead to decreased matrix synthesises and immature proteoglycan synthesis.

Such process causes joint cartilage damage \( 18 \). Previous study stated that the value of Q/H ratio did not affect the OA progress \( 19 \). Nevertheless, we found a significant difference in the Q/H ratio between both groups (\( p = 0.047 \)).

The average value of Q/H ratio in the group with stage 3 knee OA was smaller (1.969) than the group with stage 2 OA (2.68). This might be due to osteoarthritis in the early stages will cause pain, but it can reduce the patient’s mobility at a later stage \( 20 \). Decreased mobility can lead to decreased muscle strength, including quadriceps muscle. This simultaneous decline in quadriceps and hamstring muscle strength is believed to be the basis for the decrease in Q/H ratio in the group with stage 3 OA.

**Conclusion**

We found no correlation between quadriceps and hamstring muscle strength with pain level. There was no difference in quadriceps and hamstring muscle strength between groups with stage 2 and 3 OA. On the other hand, there was a positive correlation between Q/H ratio and pain level. There was a difference in Q/H ratio between groups with stage 2 and 3 OA, with average Q/H ratioin stage 3 OA was lower than stage 2 OA.

**Ethical Clearance:** The study protocol was
approved by the Ethical Commission to conduct basic science/clinical research in Dr. Soetomo Teaching Hospital Surabaya, Indonesia. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, non-maleficence, and justice.

Conflict of Interest: The author reports no conflict of interest of this work.

Source of Funding: This study is done with individual funding.

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Effect of Dates (Phoenix dactylifera L.) on Liver of Broiler Chicks Infected with Infectious Bursal Disease Virus. Biochemical and Histological Study

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Abstract

A number of (80) birds (Ross 308) at one day old were obtained from a local hatchery in the area. The birds were allotted into four equal groups (no.=20) and were supplied with basal scientific diet. Palm date (PD) was supplied to G3 and the birds were challenged with virulent Infectious Bursal Disease Virus (IBDV). G4 was treated with palm date 3 days prior to challenge age. G2 was challenged without supplementation of PD. G1 was neither given PD nor challenged. Inclusion rate of PD was 10%. The challenged age was at 33 days old. Blood serum was assayed for evaluation of transaminases and lipid profile 10 days post infection period (PIP). The result showed that histopathological investigation is more accurate in determining the health state of the birds and the palm date have to be supplied in more than 10% to give its significant effect.

Key words: Date palm, GPT, GOT, HDL, ALP, Cholesterol, Triglycerid, HDL, IBDV, Histology, Broiler Chicks.

Introduction

Cereals are an important sources of food for human and animals alike. Because of the massive population growth, the world has been faced with a gap between providing food needs and maintaining the livestock and their needs for feedstuffs. Tropical and semi-tropical regions are popular in their cultivation of palm date, which is a major source of energy. The Middle East is considered one of the world’s leading producers of dates, accounting for nearly 70% of the world’s production (1). Poultry industry is regarded as the main tool for supplying human demands of animal protein. To fulfill this requirements, grains have to be supplied and this will have negative impact on the level of human requirements. Because of dates and dates fruits are rich sources of protein and energy, the later can be used as an alternate of cereal in order to save human demands for their consumption, in addition to lower the cost of feedstuffs supplied in poultry industry (2). Many researches have shown that inclusion of palm date kernel (PDK) or seed was considerably valuable in improving productive performance, feed cost and feed utilization when added to poultry feed (2,3). The palm date has been proved to be an efficient diet during food shortage or during crises because of high nutritive value. It is rich in calcium, zinc, potassium, selenium, sulphur, chlorine, iron and manganese (4,5).

Medically, palm date have been proved in preventing liver against toxic damage produced by various toxic metabolites because of its anti-oxidant property due to high availability of various tocols as well as to its phenolic compound (6). Liver is the largest organ in the body that may show lesions represented by swelling and infarct (7) as a result of infection with infectious bursal disease virus (IBDV ) and this may have a negative impact on liver function. PDK is nontoxic and had been used against hepatotoxic and mutagenic effects, hyperlipidemia, hyperglycemic inflammation, microbial infection, oxidation and immunosuppressive effect of IBDV (8,9,10,11).

Recently, poultry industry have adopted a new strategy in poultry rearing which aimed to reduce the
marketing age through genetic selection breeding with high feed conversion ratio (FCR) and high body weight gain, palm date is said to have this property in enhancing productive performance \(^{10,12}\). For all the above mentioned reasons our study have been planned to study the effect of palm date as a medical agent in preventing liver damage caused by IBDV as well as for commercial purposes to have high productive performance.

**Materials and Method**

A total number (80) of chicks type (Ross 308) at the age of one day old were purchased from local hatchery in the area. They were divided into four sub-groups. All birds were supplied with basal scientific ration in addition to 10% whole grinded dry palm date type Zahdi (Phoenix dactylifera L.), the later was supplied at the day of challenge with exception of G4 which had been supplied with palm date three days before challenge (Table -1).

The birds were exposed to oral infection with highly virulent Infectious Bursal Disease Virus (IBDV) at the age of 33 day old. Infection was done by collection of grossly representative infected bursae with local field strain. The collected samples were homogenized, tissue suspension was centrifuged for a period of ten minutes at a rate of 2500 revolution per minute, supernatant fluid was aspirated, penicillin and streptomycin were added at a rate of two thousands IU and two mg per 1 ml respectively. Five birds from each group were sacrificed 10 days post infection period (PIP) to monitor any differences between treated and non-treated groups to evaluate blood serum related to different groups. The parameters adopted in evaluation were alkaline phosphatase (ALP), glutamic oxaloacetic acid transaminase (GOT), glutamic pyruvic transaminase (GPT), lipid profile which include cholesterol, high density lipoprotein (HDL), triglyceride (TG ) and cholesterol. The technique followed was according to kits manufacturers.

- ALP kit from Biolabo SAS, France.
- GPT and GOT kits from Randox laboratories Inc. UK.
- TG and cholesterol kits from LINEAR CHEMICALS L.U. Barcelona, Spain.
- HDL kit from Bio System S.A. Barcelona, Spain.

Histopathological study of liver was done according to Luna 1968 \(^{13}\) in order to follow the differences between treated and non-treated groups histologically.

**Distribution of chicks with their treatment.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>G2</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>G3</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
| G4     | +(3 days prior to challenge) | + |!

- : Not given palm date  
+ : Treated with 10% palm date

**Results and Discussion**

Table-2 showed that G1 (negative control) have no significant difference in GOT level when compared to G2 (171.1±34.974) which was not treated but was challenged with virulent IBDV. G2 showed high significant difference \((p<0.05)\) as compared to G3 and G4 (110.6±4.751 , 108.4±6.293) respectively. The result shown above indicated that IBDV increases the level of GOT in blood serum of IBDV infected chicks. At the same time G3 and G4 decrease significantly as compared to G1 and G2. Although G4 do not differ significantly from G3 but GOT level is lower than G3 and this may be due to reduced effect of virulent IBDV as a result of pre medication with palm date 3 days before challenge (Table -1). The study do not agree with Nadia K. J. Al-Dawah \(^{17}\) who mentioned that the level of GOT serum showed no significant differences as compared to negative control group.

**GPT** : G1 showed no significant difference from G2 and G3 (18.25 ± 0.491, 18.00 ± 1.416 ) respectively ,on the other hand the pre-medicated group (G4) (17.29±1.960) differ significantly from G1 at \(p<0.05\) level. This indicate that palm date have no effect on GPT level. The result remains questionable due to the absence of studies that support or oppose our study (Table-1).

**ALP** : The study showed that there were no significant differences between treated group and non-treated one or challenged or non-challenged (G1,G2 and G4) which may give an expression that ALP is non-indicative for the health status in poultry (Table-1). The result is in agreement with \(^{18,19}\) who mentioned that the highest level of ALP in chicken occur in the first two
weeks of life then decrease significantly with age. ALP level in G3 remains unexplained and available study to be compared.

**Table-1. 10 days PI GOT, GPT and ALP**

<table>
<thead>
<tr>
<th>Group</th>
<th>GOT Mean± SD</th>
<th>GPT Mean± SD</th>
<th>ALP Mean± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>160.3±26.068</td>
<td>21.64 ± 2.732</td>
<td>52.82 ±3.700</td>
</tr>
<tr>
<td>2</td>
<td>171.1± 34.974</td>
<td>18.25 ± 0.491</td>
<td>55.19± 0.277</td>
</tr>
<tr>
<td>3</td>
<td>110.6± 4.751</td>
<td>18.00 ± 1.416</td>
<td>10.86 ± 2.721</td>
</tr>
<tr>
<td>4</td>
<td>108.4 ± 6.293</td>
<td>17.29 ± 1.960</td>
<td>39.71± 22.028</td>
</tr>
</tbody>
</table>

Note: Different capital letters refer to significant differences between different means at 95% confidence interval.

**Cholesterol**: Table-2 showed no significant differences in the level of serum cholesterol in G1 and G4. While G2 do not differ from G3. The low level in G1 may be related to absence of challenge. Elevation of cholesterol level in the other groups could be due to the liver damage created by IBDV. Pre-treatment with PD in G4 resulted in lower cholesterol level in comparison with other infected groups (G2 and G3). This is in agreement with (20,21) who mentioned that the polyunsaturated fatty acids that compose the vegetable oils supplemented in the diet reduce both egg and blood cholesterol levels.

**HDL**: The study showed no significant differences between G1 and G2 (26.49 ± 0.899, 26.30 ± 1.349 ), (non-treated groups) on the other hand G3 and G4 (treated groups) ( 13.22 ± 5.709, 14.52 ± 9.980 ) do not differ from each others , this reflect the effect of addition of palm date to the diet, at the same time the result on HDL run in a parallel way to that effect on cholesterol (Table-2). G1 and G2 showed higher significant level than the treated groups (G3 and G4). The result agreed with (22) who mentioned that total cholesterol (TCH) level is positively correlated with HDL and LDL.

**TG**: The study showed no significant differences in different groups, treated or non-treated or challenged and non-challenged groups (Table-2). This is in agreement with (23) who mentioned that triglyceride and cholesterol concentrations were positively correlated with each other.

**Table-2. 10 days PI. Cholesterol, HDL and TG**

<table>
<thead>
<tr>
<th>Group</th>
<th>Cholesterol Mean± SD</th>
<th>HDL Mean± SD</th>
<th>TG Mean± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>57.81 ± 42.61</td>
<td>26.49 ± 0.899</td>
<td>154.2 ± 64.08</td>
</tr>
<tr>
<td>2</td>
<td>410.42 ±156.96</td>
<td>26.30 ± 1.349</td>
<td>114.0 ± 78.25</td>
</tr>
<tr>
<td>3</td>
<td>578.47 ± 15.07</td>
<td>13.22 ± 5.709</td>
<td>156.0 ± 14.76</td>
</tr>
<tr>
<td>4</td>
<td>209.17 ± 171.33</td>
<td>14.52 ± 9.980</td>
<td>190 ± 12.34</td>
</tr>
</tbody>
</table>

Note: Different capital letters refer to significant differences between different means at 95% confidence interval.
Pathological Study

The study revealed that IBDV have deteriorative effect on liver parenchyma and palm date have a great impact in reducing this effect. Transaminases are usually used to test for cellular necrosis and their increase in serum concentration may indicate liver malfunction. Liver is regarded as the site of amino acids metabolism, any elevation in blood serum may give indication that there is damage to the liver as a consequence to harmful agent that causes leakage of these amino acids to the bloodstream. This in agreement with.

G2 showed mild inflammatory cells infiltrations with congestion of sinusoid (Figure 2) as compared to control group (Figure 1). G3 showed milder pathological lesion as compared to G2 and more sever pathological lesion as compared to G4, this might be due to previous medication with palm date (Figures 3 and 4).

The study revealed that there is no correlation between serological tests and histological findings. G1 and G2 showed lower significant level of GPT in spite of sever pathological lesions in G3 and G4 as compared to the control group. The result remains unexplained because of no available comparative study. Most high triglycerides are associated with an increase in LDL cholesterol and decrease in HDL cholesterol also there are many factors that estimate the level of lipid profile as the sex, age, abdominal fat. This support our study that histological investigation is more indicative to determine the health status of the bird.

The study showed that IBDV have no effect in the level of TG and ALP, this is because of the positive relationship between triglyceride and cholesterol concentrations which are positively correlated with each other.

The study concluded that palm date may reduce the harmful effect of infectious agent as IBDV due to its high component of active anti-oxidants that playing an important role in neutralization of free radicals and decomposition of peroxides.

Conclusion: that the addition of 10% palm date may give no indicative serological parameters for the health status of the bird; Hisopathological investigation still remains the only way for evaluation the health status of birds.
Figure 2: G2. liver showed fatty degeneration with coagulative necrosis of hepatocytes and inflammatory cell infiltration H and E 400X.

Figure 3: G3. Treatment after challenge. Inflammatory cell infiltration) and hepatocyte atrophy with sinusoid congestion .H and E 400X.
Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References


Identifying the Correlation between the Incidence of Breast Cancer and Hepatitis B Virus of Iraqi Women

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Abstract

Objective: Women are half of the Iraqi community and their health should be priority to the country. Breast cancer occurs increasingly rising in Iraq due to so many problems that the country has suffered. Several previous studies have studied breast cancer; however, none of them has explored the presence of HBV and the basic molecular mechanism are generally obscure and research studies are not enough and disintegrated.

Method: Therefore, this study are focusing on discovering a novel technique to detect breast cancer as early as possible to kept women lives. Advancement in routine breast cancer risk assessment will prevent chemotherapy uptake in women. Consequently, the women with or without breast tumor have expose to plasma assay to reveal HBV, Estrogen, Progesterone, complete blood count. The women test are divided into many classes of such as age, smoke and non-smoke to test their ability to detoxify carcinogenic aromatic amines in cigarette smoke.

Result: 64% of the malignant women were smoker while 36% were none smoker. 45±12 years is the most vulnerable age to get cancer. HBsAg were demonstrated in 10% of the all malignant ladies, while anti-HBs Ag have detected in only 5% of the total number. Complete blood counts and hormonal levels were dysregulated in the breast cancer patients.

Conclusion: The hormones are negatively correlated with the presence of cancer, HBV have detected in several samples but the no significant correlation. Smoking is the most factor significantly associated with breast tumor. For all these reasons. Iraqi government strongly argue to make smoking cigarettes prohibited in public areas.

Keywords: breast cancer, progesterone, estrogen, HBV, smoking

Introduction

International Agency for research cancer (IARC) has reported that female breast cancer is responsible about 11.6% of total cancer incidence, and documented to be a common diagnosed cancer and ranked as number one leading death for women worldwide (1). Fortunately, breast cancer is highly curable if diagnosed at earlier stage, but it might be deadlier if discover at later stages (2). Breast cancer (BC) is a heterogeneous illness ranked as the highest happening of all females cancer types and number leading death worldwide (3). In 2015, 143 cases were registered as positive breast cancer in Thi-Qar represented by 11.7% off all cancer cases registered in the city. In Asia, and particularly in the Middle East there is a rising incidence of breast cancer even though they migrate to western countries years ago (4). Contrary to the latest drop in the breast cancer occurrence in Western nations in comparison the occurrence in Iraq has been increasingly rising. The estimated number of Iraq’s population in 2015 is 36,933,714 included 18,659,573 males and 18,274,141 females (Ministry of planning / Central Statistic Organization). The number of breast cancer in Iraq which is 3763 cases in 2011 as it recorded in the cancer registry data published by the Iraqi cancer board, has reached a scary number in 2015 so breast cancer raised to be second female mortal cancer in Iraq. These documents ring the bell for imminent step towards understanding the epidemiology and the reason that underline the breast cancer predisposition. The expanded rate of the this kind of sickness could be due to other trends such as lower parity, postponed childbirth, declined breast feeding, war, terrorist that reduce the
health care services and sedentary lifestyle. The survival average of breast tumor are determined by the stage of diagnosis, therefore, there is increasing occurrence of breast cancer doom for women living in the rural in comparison to women living in the urban area.

There were no obvious examples of expanded hazard related with smoking by N-Acetyltransferase 2 (NAT2) state among premenopausal ladies. In postmenopausal ladies, who are slow acetylators, NAT2 strongly changed the relationship of smoking with breast cancer risk. These data strongly support this hypothesis that discovering and detoxifying carcinogens accumulated in the body from environment might reduce the breast cancer mortality rate.

On the other hand, genetics variations are an earlier signs of breast cancer such as P53, ZHX1, 2 and 3 genes. NAT1 and NAT2 the major decrease in breast malignant growth has managed by a full term pregnancy recommends the extraordinary capability of preventive hormonal procedures. As opposed to the dangers related with delayed exposures, exogenous estrogen and progesterone for brief terms can copy the defensive impacts of pregnancy in cancer-causing agent actuated mammary tumor models. About 300 million individuals worldwide affirmed positive for Hepatitis B infection (HBV) as well as it is a basic cause of hepatic disease and hepatocellular carcinoma. HBV is an individual from the Hepadnaviridae family that incorporates A, C, D, E. HBV is a little DNA infection with uncommon characters practically identical to retroviruses and delegated Orthohepadnavirus family has a size 3.2 DsDNA genome that is secured by HBsAg. The clinical significance of the infection originates from its capacity to cause unending illness and being oncogenic. HBV reiterate by means of RNA moderate and can incorporate into the host genome. The special portrayals of the HBV replication cycle present a particular capacity of the infection to endure in infected cells. serological parameters and sub-atomic examines have been improved for the identification of various sorts of HBV connected malady and for relieving of endless hepatitis B contamination. HBV contamination is a noteworthy motivation to a wide range of liver sickness. The hepatic injuries a

Are ranging from intense such as counting fulminant hepatic disappointment to constant hepatitis, cirrhosis, and hepatocellular carcinoma. Intense HBV was contaminated human could be superbly fine or show side effect of intense hepatitis. Most adults polluted with the infection recover, while 5%–10% are unfit to clear the inflammation and become endlessly diseased. Numerous incessantly tainted people have mild liver sickness with practically no morbidity or mortality. Other individuals with chronic HBV infection may continue to compelling illness, which can continue to cirrhosis and liver malignant growth. These patient’s supplication demanding observing and remedial mediation. Extra hepatic indications of HBV disease are uncommon yet can be hard to identify and treatment. The challenges in the field of HBV illness are the poor instruments in anticipating result and movement of HBV disease and the stubborn need to comprehend the cell, immunological, furthermore, acquired premise of different ailment displays related with HBV disease.

Material and Method

Samples:

150 women have involved in the study from the main teaching hospital in Thi-qar province, 100 specimens were clinically and histopathology examined and reported to have malignant breast cancer approved via clinical examination, mammographic screening, breast ultrasound, breast magnetic resonance imaging (MRI). our study has included women have suffered breast shaping alteration characterized by the presence of hard, fixed, breast lumps and/or swollen breast of one or both sides. one-sided nipple ejection mainly bloody discharge with or without palpable lump are the chief signs of women included in my study. As the age is being the main risk factor, the patients were divided into age groups.

On the other hand, this study have involved 50 ladies were undergone breast biopsy taken, but the oncologist has reported them to be either having benign tumor or none at all so them have used as a control group. Smoking mean the lady is a smoker or live with a partner who is a smoker, non-smoking means the lady is neither she or her household smoke.

Serological tests by ELISA:

Hepatitis B infection is the most hideous kind of hepatitis; hence, I centered my examination on the estimations of hepatitis B infection antibodies and antigens in the patient’s blood. To determine the period of illness whether it is intense or ceaseless; for
all patients; it utilized HBs Ag to identify the hepatitis Ag altogether, followed by testing HBc to determine the antibodies for the center antigen for all cases by ELISA (Bioprobes - Italy). Biochemical tests were utilized to distinguish the liver protein ALT Alanine transferase (GPT) to measure the hepatic cell damage.

**physiological parameters:** These investigation have included complete blood count has been done for all the samples as well as hormonal measurements have been done by collecting the blood from the patients in the middle of their menstrual cycle. Minvidus device has been used to measure the hormones.

**Statistical analysis:**

GraphPad crystal variant 7 programming for Windows, La Jolla California USA, www.graphpad.com, it was utilized to average and plot the information in this project with standard deviation. Although performing t-test between two groups, crystal was utilized ANOVA to break down at least three bunches pursued by numerous correlations utilizing Tukey’s test. Significant information designed by star as the following: 0.01 ≤ *p <0.05; 0.001 ≤**p <0.01; ***p <0.001.

**Results**

The 100 ladies were malignant were divided into 64% to be smoke that term include an active or second-hand smoke. If the woman is an active smoke means her is the person who smoke or live in the smoking house or work at smoking environment, while 36% were not smoking women (Figure 1). The incidence of the disease where around the age of 45±12 to be the significant age to have malignant disease (Table1).

Only 10% have shown positive HBs Ag, while 90% were negative (Figure 2). On the other hand, there were 5% of the breast cancer ladies shown positive Anti-HBc antibodies while 95% were negative.

Complete blood count was normal for control group and low for the cancer group including WBC, RBC, Platelets (data not shown). Hormone test has shown normal level Progesterone for the normal group, it was around 5-20 ng/ml, while normal estrogen was 15-350 pg/dl for the pre-menopausal women and 10 pg/dl for the post-menopausal ladies (Table 2).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Benign tumor N 50</th>
<th>Cancer 100</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>50± 10</td>
<td>45± 12</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>HBV Positive</td>
<td>5</td>
<td>18</td>
<td>Not significant</td>
</tr>
<tr>
<td>HBV Negative</td>
<td>45</td>
<td>82</td>
<td>Not significant</td>
</tr>
</tbody>
</table>

![The percentage of smoke and non smoke study patients](image)

**Figure 1:** Breast cancer patients classified into smoke and non-smoke categories.
Figure 2: The correlation between HBs Ag and breast cancer

Figure 3: The correlation between Anti-HBc antibodies and breast cancer

Table 2: Hormones test were performed to the patients in the projects

<table>
<thead>
<tr>
<th>Patients samples</th>
<th>Pre-menopausal</th>
<th>Post-menopausal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone</td>
<td>15±5 ng/dl 60%</td>
<td>0.3-1 ng/dl 39%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.0 ng/dl 1%</td>
</tr>
<tr>
<td>Estrogen</td>
<td>200±15 pg/dl 60%</td>
<td>10 pg/dl 30%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>150 pg/dl 10%</td>
</tr>
</tbody>
</table>
Discussion

The estimated number of Iraqi populations is 2,029,345 in Thi-qar Province and 7,877,888 in Baghdad city.

My project will significantly contribute to science since it examined the alteration with the presence or absence of breast cancer and link this alteration to the environment whether it is smoking or not smoking area. I presume detecting a dose response correlation between the number of cigarettes smoked and the breast cancer risk but unfortunately, it could not measure the correlation in the present project.

The full epidemiological features of breast cancer in Iraqi women are not yet fully understood. My project is novel since it aims to discover a novel method that could detect breast cancer.

There was a significant correlation between the smoked life style and the breast cancer incidence. The results are consistent with several research across the world (5,8) who found that there is a link between the carcinogenic materials in the cigarettes. In my opinion, carcinogenic materials can dispose in the human body. Then, with recurrent exposure, DNA mutation might occur and could be followed by second hit mutation to end up forming malignant mortal cancer.

Hepatitis B disease is prevalent in Iraq. So, the result 10% and 5% only infected patients were surprising since it expected more due to the epidemic virus infection. These data could be explained with the low number of patients so 100 patients is not enough to get the epidemiological study. The present study has recommend to use 500-1000 cases across the 17 provinces of the country to do a reliable research enough to get scientifically meaningful conclusion.

This study has done a case-control project and compared WBC, RBC counts between patients with breast cancer and tendency score-matched controls. Laboratory test has been examined such as complete blood count, the complete blood count WBC were in normal range from 3,700 to 10,500 for the healthy control group and a decreased WBC count in women could have undiagnosed breast cancer or may be due to chemotherapy for individuals already have cancer. It could be low due to a viral infection, a toxic reaction, or a process in the bone marrow that restrictions the body’s ability to create normal WBCs.

Hormonal tests have shown a high level of progesterone and Estrogen for pre-menopausal in women under 50 years old are expected. On the other hand, women older than 50 years or post-menopausal have expressed lower hormonal levels and was expected as it the normal physiology of the women body (Table 2).

Few cases have shown normal hormonal levels, after investigation, it appears that their doctor has prescribed medical hormones treatment which play principal roles in coordinating suitable growth and function of breast tissue. High levels of these hormones are correlated with pregnancy and seem to be responsible for the weakened risk for breast cancer among women following a full-term pregnancy. The patients samples are classified as stage I or stage II when the tumor size is £ 4cm according to (11).

This current study may be assess of the samples by smoke/non-smoke, ER/PR status, menopausal status, and body mass index (M. Al-Kafajy unpublished data) and weighed the association between WBC, RBC count, genetic expression and breast cancer encumbrance using multinomial logistic regression.

The work was aimed to set standards of breast cancer risk prognostication by analysis the alteration of gene expression and particularly spotted the specific genomics aberration in Iraqi women such as BRCA1, BRCA2, HER2/ neoucogene, progesterone and estrogen receptors, P53, ZHX1, 2 and 3 genes. Genetic alteration in P53 is not expected except in the ladies who have cancer since it is the guardian of the cell. Alteration in P53 will be anticipated in the samples that already have cancer. ZHX1, ZHX2 and ZHX3 could be altered in the expression or protein concentration or localization (12). Potential application for the project will be forcing a healthy habit for the individual and set laws that protect second hands smokers from the carelessness of most of the smokers.

Future Direction

Our study like to expand present project to examine the smoking duration and its ongoing change in the physiological and genetic level as it already have been done in the western countries and found a modest relationship in the pre and post-menopausal women (8). Our team would like to expand my project to include women from areas know to be contaminated such as factory region in Baghdad which is known to
be environmentally bad area to live in. On the other hand, the area that were under terrorist occupation has contaminated with war products and probably the women they don’t have access to suitable health care. Moreover, the refugee camps in the Kurdistan area would be another spot that could be included in the study since the poverty rate and hard circumstance in this area make it really difficult to have a good health care system.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** Self-funding

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12. Al-Kafajy M. Expression of Zinc Fingers and Homeoboxes 2 (Zhx2) and Zhx2 Target Genes in Multiple Tissues of Wild-Type and Zhx2 Knockout Mice. 2016.
Cyclic Fatigue Resistance of Wave One Gold, F6 SkyTaper, One Curve, and AF Blue R3 NiTi Rotary Instrumentation Systems

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Abstract

Objective: This in vitro study is aimed to compare and evaluate the cyclic fatigue of four varying NiTi rotary instrumentation systems.

Method: In this study, four types of rotary files were used in four groups (10 files for each group), namely, Group A: Wave One Gold; Group B: AF Blue R3; Group C: One Curve; Group D: F6 SkyTaper. These groups were evaluated by a cyclic fatigue apparatus to measure cyclic fatigue resistance within the artificial metallic simulating canal that has a 60° angle of curvature, the curvature radius was 5 mm, whereas the inner diameter of the canal was 1.5 mm. All the files were rotated in artificial canals until they fracture. The resistance to cyclic fatigue was determined by counting the number of cycles to fracture, and the time to failure was recorded in seconds then transformed to minutes. In addition to that the fractured fragment length was evaluated.

Results: One-way analysis of variance test showed a significant difference (P<0.001) in the average values of cycle number and time needed for file fracture and average fractured portion length among all groups. The t-test results indicate a significant difference (P<0.001) among all groups.

Conclusions: The study concluded that with an apical curvature of an artificial canal with an angle curvature of 60°, AF Blue R3 and F6 SkyTaper instruments exhibit a higher resistance to cyclic fatigue than One Curve and Wave One Gold files most possibly due to surface and alloy feature variation.

Keywords: Endodontics instrumentation, cyclic fatigue resistant

Introduction

Rotary instruments for root canal systems made from nickel-titanium (NiTi) alloy exhibit more elasticity than stainless steel instruments, which are rigid and unsuitable for large apical enlargement in thin-curved canals [1]. Specifically, the elastic flexibility of NiTi instruments is approximately 2–3 times greater than that of stainless-steel instruments [2&3].

NiTi instruments feature a risk for fracture owing to cyclic fatigue and torsional shear; this condition is an unexpectedly important disadvantage of rotary files when rotate freely in a curved root canal [4-6]. In order to increase the easiness, simplicity, and speed of root canal preparation procedure for practitioners, a single file Ni Ti rotary systems with either complete rotation or reciprocation motion were introduced [7]. Wave One Gold files manufactured by Dentsply Maillefer in Switzerland are a new version of Wave One files. In this version, the geometry, dimensions, and cross section are modified while maintaining reciprocation motion. The files are made by gold, which is heated at first and then slowly cooled for file production; these files also exhibit two cutting edges with a parallelogram cross section, which may increase file flexibility as claimed by the manufacturer [8].
The F6 SkyTaper manufactured by Komet, Brasseler GmbH & Co. in Germany is a new generation of single-file, one-use NiTi system comprising one available instrument with five diverse sizes (20, 25, 30, 35, and 40) and showing a constant taper of 0.06, which is critical for shaping of the root canal. These files are distinguished by their cross-sectional design, which is a unique double-S cross section. The F6 SkyTaper instrument is produced for use in a continuous clockwise rotation motion [9].

One Curve file manufactured by MICRO MEGA in France is a heat-treated C-wire with a regulated memory of NiTi and a potential to prebending to facilitate access to the root canal. One Curve is also a single-use, single instrument rotary file employed in continuous rotation. The variable cross-section ensures excellent cutting efficiency and debris removal up to the medium and coronal parts of the canal with a perfectly centered trajectory [10&11].

AF Blue R3 manufactured by Fanta Dental Materials Co., Ltd. in Shanghai is a special heat-treated wire used for producing endodontic rotary files. However, NiTi files display a high possibility of unnoticed fracture inside root canals, different from the stainless-steel files, which are most likely to show plastic deformation signs. AFTM-Wire is a developed NiTi alloy and features excellent mechanical strength properties. The flexibility of AFTM-Wire sufficiently avoids canal transportation. Meanwhile, its hardness is large enough to allow for good cutting efficacy. Fanta AFTM-Wire offers three levels of flexibility depending on the crystallographic phases present in the alloy and rectangular cross-section of AF BLUE R3 [12].

The null hypothesis indicates that the tested rotary NiTi instruments show no differences in their cyclic fatigue resistances.

This in vitro study is aimed for comparison and evaluation the cyclic fatigues of Wave One Gold, F6 SkyTaper, One Curve, and AF Blue R3 NiTi rotary instrumentation systems.
In this article, four different rotary instruments were used, and 10 instruments for each type were investigated in the four groups.

**Group A:** Wave One Gold (primary, #25.07), 25 mm length, and NiTi rotary instruments (350 rpm/5 N cm).

**Group B:** AF Blue R3 file (#25.06), 25 mm length, and NiTi rotary instruments (300 rpm/2.6 N cm).

**Group C:** One Curve (#25.06), 25 mm length, and NiTi rotary instruments (300 rpm/2.5 N cm).

**Group D:** F6 SkyTaper (#25.06), 25 mm length, and NiTi rotary instruments (300 rpm/2.2 N cm).

The instruments were tested within an artificial simulating canal (angle curvature: 60°; Figure 1). The curvature radius was 5 mm, whereas canal width was 1.5 mm. The simulated canal constructed within stainless-steel blocks covered by a swiveling glass allowed for observation of files rotating in the canal and discard of broken instruments after each test [13].

To eliminate binding in the simulating canal, the files were created to be slightly wider than those used in the test (0.3 mm wider than the instruments used), granting a modest lateral movement inside the canal [14&15].

The cyclic fatigue testing apparatus utilized in this study, as shown in Figure 2, was previously illustrated. The apparatus consists of a wood main frame attached with an electric hand piece and a simulating canal carved in a stainless-steel block. The electric hand piece is supported to a movable apparatus, allowing accurate and repeated placement of each instrument inside the simulating canal [16].

The testing canal was filled with a lubrication medium (3-In-One Multi-Purpose Oil manufactured by WD-40, USA) to minimize the friction produced by the tested file with the canal walls [17]. A glass cover was fixed by the clipper to facilitate file insertion inside the canal and to prevent the instrument from slipping out, thus also providing a clear view of the instruments.

A button on the electric motor was pressed to initiate rotation. Meanwhile, a digital stop watch was also operated. Instrument rotation was monitored by the operator until the file fractured, and the corresponding time was recorded. The electric motor button was pressed again once to stop rotation during fracture of the instrument. The slide was opened, and the fractured file was replaced by a new one.

The time needed for instrument fracture from the beginning of the rotation was recorded in seconds then transformed into minutes by dividing over 60. Afterward, time (T) in minutes was multiplied by revolutions per minute (RPM) to conclude the number of cycles or rounds needed for each instrument to fracture (NCF) as shown in the following equation:

![Fractured fragment (mm)](figure4.jpg)
The length of fractured fragments for individual instruments was attained by measuring the instrument length after fracturing using a digital Vernier caliper (measured from the end of the handle to the fractured tip). The values were then subtracted from the file length before fracturing (25 mm), as shown in the following equation:

\[ \text{Fractured fragment} = \text{File length Before fracture (25 mm)} - \text{File length after fracture}. \]

Statistics, including maximum, mean, minimum, and standard deviation (S.D.), calculated for the cycle numbers needed to fracture each file. The data were obtained and noted using SPSS (program version 18) and used in statistical analysis.

One-way analysis of variance (ANOVA) was utilized to determine any statistical difference between the mean cycles needed for fracture occurrence for the different rotary instruments. A separate t-test was performed to assess the significance of variance between a pair of instruments.

In the present test, P values of more than 0.05 were considered statistically non-significant, whereas P values equal or less than 0.05 were regarded as significant. On the other hand, P values less than 0.01 were regarded as highly significant.

### Results

Table (1): Descriptive Statistical analysis for the time (Seconds) and number of cycles to fracture for groups

<table>
<thead>
<tr>
<th>Total group</th>
<th>Type of test</th>
<th>Min</th>
<th>Max.</th>
<th>Mean</th>
<th>±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A Wave One Gold</td>
<td>Time</td>
<td>108.42</td>
<td>113.13</td>
<td>110.94</td>
<td>1.870</td>
</tr>
<tr>
<td></td>
<td>No. of cycles</td>
<td>632.45</td>
<td>659.92</td>
<td>647.18</td>
<td>10.908</td>
</tr>
<tr>
<td>Group B Blue R3</td>
<td>Time</td>
<td>190.79</td>
<td>214.89</td>
<td>207.66</td>
<td>10.105</td>
</tr>
<tr>
<td></td>
<td>No. of cycles</td>
<td>953.95</td>
<td>1074.45</td>
<td>1038.31</td>
<td>50.527</td>
</tr>
<tr>
<td>Group C One Curve</td>
<td>Time</td>
<td>114.14</td>
<td>121.82</td>
<td>118.91</td>
<td>2.900</td>
</tr>
<tr>
<td></td>
<td>No. of cycles</td>
<td>570.7</td>
<td>609.1</td>
<td>594.59</td>
<td>14.500</td>
</tr>
<tr>
<td>Group D F6 SkyTaper</td>
<td>Time</td>
<td>152.13</td>
<td>164.21</td>
<td>159.06</td>
<td>5.189</td>
</tr>
<tr>
<td></td>
<td>No. of cycles</td>
<td>760.65</td>
<td>821.05</td>
<td>795.3</td>
<td>25.946</td>
</tr>
</tbody>
</table>

Table (2): ANOVA test for the time and number of cycles to fracture for groups

<table>
<thead>
<tr>
<th>Type of test</th>
<th>S. O. V.</th>
<th>SS</th>
<th>Df</th>
<th>MS</th>
<th>F-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to fracture</td>
<td>Between</td>
<td>47166</td>
<td>3</td>
<td>15722</td>
<td>715.67</td>
<td>0.000 HS</td>
</tr>
<tr>
<td></td>
<td>Within</td>
<td>615</td>
<td>28</td>
<td>21.968</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>47781</td>
<td>31</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of cycles to fracture</td>
<td>Between</td>
<td>947894.2</td>
<td>3</td>
<td>315964.7</td>
<td>568.61</td>
<td>0.000 HS</td>
</tr>
<tr>
<td></td>
<td>Within</td>
<td>15558.9</td>
<td>28</td>
<td>555.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>963453.1</td>
<td>31</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P<0.001 Significant
Table (3): t-test for the difference in time and number of cycles to fracture between each two groups

<table>
<thead>
<tr>
<th>Comparison Groups</th>
<th>t-test time to fracture</th>
<th>p-value</th>
<th>t-test number of cycles</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A &amp; Group B</td>
<td>19.240</td>
<td>0.000</td>
<td>15.280</td>
<td>0.000</td>
</tr>
<tr>
<td>Group A &amp; Group C</td>
<td>5.165</td>
<td>0.007</td>
<td>6.480</td>
<td>0.003</td>
</tr>
<tr>
<td>Group A &amp; Group D</td>
<td>16.821</td>
<td>0.000</td>
<td>9.996</td>
<td>0.001</td>
</tr>
<tr>
<td>Group B &amp; Group C</td>
<td>19.884</td>
<td>0.000</td>
<td>19.884</td>
<td>0.000</td>
</tr>
<tr>
<td>Group B &amp; Group D</td>
<td>13.546</td>
<td>0.000</td>
<td>13.546</td>
<td>0.000</td>
</tr>
<tr>
<td>Group C &amp; Group D</td>
<td>13.616</td>
<td>0.000</td>
<td>13.616</td>
<td>0.000</td>
</tr>
</tbody>
</table>

*P<0.05 significant **P<0.001 High significant ***P>0.05 Non significant

Table (4): Descriptive Statistical analysis for the length of fracture fragment (mm) for each group

<table>
<thead>
<tr>
<th>Groups</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>2.02</td>
<td>3.30</td>
<td>2.90</td>
<td>0.475</td>
</tr>
<tr>
<td>Wave One Gold</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group B</td>
<td>3.63</td>
<td>5.30</td>
<td>4.25</td>
<td>0.554</td>
</tr>
<tr>
<td>Blue R3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group C</td>
<td>2.42</td>
<td>3.04</td>
<td>2.70</td>
<td>0.216</td>
</tr>
<tr>
<td>One Curve</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group D</td>
<td>4.49</td>
<td>5.81</td>
<td>5.01</td>
<td>0.408</td>
</tr>
<tr>
<td>F6 SkyTaper</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (5): ANOVA test for the length of fracture fragment among groups

<table>
<thead>
<tr>
<th>S. O. V.</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
<td>29.211</td>
<td>3</td>
<td>9.737</td>
<td>52.349</td>
<td>0.000</td>
</tr>
<tr>
<td>Within</td>
<td>5.229</td>
<td>28</td>
<td>0.186</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>34.441</td>
<td>31</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P<0.001 Highly Significant.
Table (6): t-test for the length of fracture fragment between each two groups

<table>
<thead>
<tr>
<th>Comparison Groups</th>
<th>t-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A &amp; Group B</td>
<td>8.293</td>
<td>0.000</td>
</tr>
<tr>
<td>Group A &amp; Group C</td>
<td>0.854</td>
<td>0.421</td>
</tr>
<tr>
<td>Group A &amp; Group D</td>
<td>7.430</td>
<td>0.000</td>
</tr>
<tr>
<td>Group B &amp; Group C</td>
<td>6.425</td>
<td>0.000</td>
</tr>
<tr>
<td>Group B &amp; Group D</td>
<td>2.885</td>
<td>0.023</td>
</tr>
<tr>
<td>Group C &amp; Group D</td>
<td>15.617</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 1 summarizes the descriptive statistics for each system. The average cycle-to-fracture values of the AF Blue R3 group were the highest among all groups, whereas the lowest was observed in the Wave One Gold group.

The data inspected by one-way ANOVA demonstrated considerable variation (P<0.001) in the NCF mean values among all groups, as shown in (Table 2).

Using t-test, further comparisons among groups were conducted to determine where the significant difference occurred, as shown in (Table 3). The t-test results reveal a considerable variation among all groups (P<0.01), except for Group A (Wave One Gold) and Group C (One Curve) (P< 0.05), where is not a statistically significant differences in variation were reached (P=0.05).

Table 4 lists the mean, minimum, maximum, and S.D. of the fractured fragment length for each rotary instrument tested. The highest mean value of fractured fragment is noted in group D.

From the ANOVA test, as shown in (Table 5), a statistically significant difference was observed in the length of fractured fragments among the instruments.

T-test was utilized to explain the statistically significant difference in the fractured segment length of among the rotary instruments. A considerable difference was found among Wave One Gold, AF Blue R3, and F6 SkyTaper instruments, whereas a nonsignificant difference was observed in the One Curve group. Meanwhile, the AF Blue R3 group showed a significant difference in fracture length when compared with the One Curve and F6 Sky Taper groups. The One Curve group displayed a significant difference compared with the F6 SkyTaper group (Table 6).

The F6 Sky Taper group revealed the longest fractured segment length, followed by AF Blue R3, Wave One Gold, and One Curve groups.

**Discussion**

The fracture of rotating NiTi instrument is one of the most critical complications that develop during root canal preparation [18]. Diverse classes of rotary NiTi instruments show variation in resistance to fatigue failure owing to differences in numerous factors, such as their manufacture [18&20].

In this study, the resistance of rotary files to cyclic fatigue was tested under simulating conditions to reduce the effect of other failure mechanisms, e.g., canal diameter and length. Similar to other studies, the test was evaluated using stimulating canals made of stainless steel with a radius of 5 mm and 60° curvature [21&22]. Considering that this study aimed to examine the physical properties of NiTi rotary files, the extracted teeth were unsuitable models as no two root canals are exactly identical [23]. One operator has examined the files, whereas the other operator has operated the stopwatch [24]. The Wave One Gold, AF Blue R3, One Curve, and F6 SkyTaper NiTi rotary instrumentations were selected given their differing cross sections, processing metallurgic alteration, and rotation axes. Comparisons were conducted with files of similar curvature and diameter to minimize the confounding variables. This study recorded the fractured fragment mean length of the files to ensure exact positioning of the tested files inside
the simulated canals and to determine whether stresses were induced at the canal curvature. The Blue R3 files were the most resistant to cyclic fatigue, followed by F6 SkyTaper, One Curve, and Wave One Gold files. The t-test results showed a significant difference among all groups (P<0.01). However, the difference between Wave One Gold and One Curve (P<0.05) groups showed no statistical significance. In the AF Blue R3 files, the cyclic resistance may be related to a more metal mass and a larger cross-sectional area due to its rectangular cross section in addition to the AF-R Wire Technique, which depend on the crystallographic phases present in the alloy. The files are manufactured with NiTi, which undergoes an innovative heat treatment, altering its molecular structure, to achieve an increase in cyclic fatigue resistance; surface treatment of the file involves chemical polishing, and additional flexibility and a distinct blue color are also added [12]. However, no data were published until the date of evaluation of the cyclic fatigue resistance of the roots by using AF Blue R3 files. Therefore, comparison of our results with other studies is impossible. The F6 SkyTaper instruments showed more cyclic fatigue resistance compared with the One Curve and Wave One Gold instruments. This observation may be related to the decreased cross-sectional area associated with the unique double S-shaped cross-section design of F6 SkyTaper. Plotino et al. noted an inverse relation between cyclic fatigue resistance and cross-section metal mass of NiTi files [25]. This result agrees with that of a study concluding that Wave One Gold shows low resistance to cyclic fatigue [26&27]. This difference in the cross section between files might also contribute to their cyclic fatigue resistance.

Wave One Gold instruments were less resistant to cyclic fatigue and were fractured within a short period possibly due to their cross-section design (parallelogram-shaped cross section), which should be regarded for conclusions of this study. The thermal processing increased the temperature of austenitic transformation of nickel titanium alloy and improved its crystal structure arrangement [28&29]. These factors may improve instrument performance. One Curve files are manufactured with a C-wire by applying controlled heat treatment with the property of regulated memory, thus improving file resistance to cyclic fatigue. Parashos et al. noted that small core diameter enhances instrument resistance to cyclic fatigue [30]. One Curve features a low core diameter (approximately 48.327 μm²). All these findings explain the enhanced fatigue resistance of One Curve files. The instrument morphologies and geometries, such as the cross section design and core diameter, may be considered significant determinants of the position of fractured fragments of files on the curvature of root canal.

Conclusion

Considering the outcomes in this article, we can conclude that AF Blue R3 file exhibits the highest resistance to cyclic fatigue in comparison with F6, One Curve, and Wave One Gold files. Further research is advised to assess the resistance to cyclic fatigue of these files by using different canal lengths and curvatures. The clinical performance of these new files should also be evaluated.

References


[29] Plotino G, Grande NM, Cotti E, Testarelli L, Gambarini G. Blue treatment enhances cyclic fatigue resistance of vortex nickel-titanium rotary

Relation between Cardiorespiratory Fitness Measured with Six-Minute Walk Test and Walking Speed Measured with 1—
Meter Walk Test in Patients of Post-Subacute and Chronic Ischemic Stroke

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Abstract

Background: Cardiorespiratory fitness measurement using 6MWT (Six Minute Walking Test) and walking speed measurement using 10MWT (Ten Minute Walking Test) in post-stroke patients are measurement to assess post-ischemic stroke patients in subacute or chronic phase. This study is to assess cardiorespiratory fitness and to measure walking speed of post-stroke patients in subacute and chronic. We also aim to observe the relation between cardiorespiratory fitness and walking speed in post-stroke patients.

Method: This study involved 38 subjects as samples. 6MWT assessment was conducted by measuring the mileage in six minutes. Walking speed was conducted afterwards with self-speed and fast-speed which were respectively assessed through three repetitions.

Results: We found string correlation in 6MWT assessment results with mileage in meter and VO2 max (r = 0.680 and p = 0.000). The result of 6MWT measured with VO2 max (ml/kg/minute) indicated strong correlation with 10MWT both with self-speed (r = 0.715, p = 0.000) and fast-speed (r = 0.687 p = 0.000).

Conclusions: We concluded that there was a relation between cardiorespiratory fitness and walking speed in post-subacute and chronic ischemic stroke patients.

Keyword: cardiorespiratory fitness, stroke, walk test.

Introduction

In post-stroke, continuous aerobic capacity usually occur in acute phase which reduce cardiorespiratory fitness reserve 1. In addition, the high amount of energy required for walking as well as comorbidities lead to functional limitations for this population 2. Cardiorespiratory fitness in post-stroke decreases from 50% to 70% depends on certain ages and genders. In elderly, the decrease occurs more rapidly. Cardiorespiratory fitness also decreases faster in female than in male. The sharpest decline is up to seven weeks post-stroke. The energy level required by stroke patients for routine ambulation is 1.5-2 times higher than in healthy individuals which indicates 76% of their physiological capacity 3.

This is due to post-stroke muscle fatigue and low level of cardiorespiratory fitness related to declining functional activity performance. Many studies suggested that it is associated with low peak oxygen consumption (VO2peak) 3. The average peak oxygen (VO2peak) consumption in post-stroke patients is around 11.4-17.3 mL/minute. This is 50-60% of VO2peak in healthy individuals in accordance with age and gender 4. Maximal oxygen uptake (VO2pMax) decreases to 10-17 mL/kg/minute during 0-30 days post-stroke 5.
Patterson et al. found that short-distance walking in chronic stroke patients is related to balance, cardiorespiratory fitness and weak side limb strength. The difference of ability in long-distance walking is caused by severe sensory fatigue and deficit which lead to poor gait. Balance also becomes an important factor for those who walk slower, while cardiorespiratory fitness plays bigger role in those who walk faster.\(^6\)

Janice et al. also conducted a study in chronic stroke patients and reported that stroke specific impairment was the main obstacle for chronic stroke patients to take longer distance. In the study, they found that the subjects’ heart rate in the study reached steady state after six minutes of walking. This indicates that six-minute walk test (6MWT) may depict cardiorespiratory fitness. Six-minute walk test (6MWT) also strongly correlates with self-paced walking speed.\(^7\)

There is a study on subacute stroke patients conducted by Kelly et al. suggesting that 6MWT is strongly related to peak cardiorespiratory fitness and also strongly correlated with self-paced walking speed.\(^8\)

Walking speed assessment can also be conducted using 10 meter walk test (10MWT), a time measurement required to do 10-meter walking with patients’ maximum walking speed. The test began with three minutes of warming up. Walking speed is the parameter of disability in walking fast and easily which is recommended as a measuring instrument in stroke rehabilitation.\(^9\)

It is still debatable whether or not there is a correlation between walking speed, walking distance taken by the subjects, functional walking test (including 6MWT) and aerobic capacity in post-stroke patients. For instance is a conclusion from a study conducted by Tang et al. stating that even though 6MWT might be able to spur cardiorespiratory system, the results seem to be more affected by walking ability limitations than by cardiorespiratory capacity.\(^2\)

In this research, the authors aim to find out if there is a relation between cardiorespiratory fitness level using 6MWT measuring instrument and walking speed in post-ischemic stroke patients and if walking speed in all ischemic stroke phase is related to cardiorespiratory fitness level. Such study has never been conducted before in Dr. Soetomo General Hospital Surabaya.

### Methods
This was an observational analytic study with cross-sectional design. It was conducted from July to October 2014 in Medical Rehabilitation Installation of Dr. Soetomo General Hospital Surabaya. The samples of this study were post-ischemic stroke that met the inclusion criteria (post-hemiparesis ischemic stroke patients, more than 2 weeks until 1 year post-ischemic stroke, adult age of 30 to 65 years old), able to independently walk the distance of 60 meters for six minutes with or without aids, no complains of pain, asphyxiate, heart-pounding and cold sweat, able to conform with simple commands such as “start”, “stop” and “you may rest if you are tired”) and exclusion criteria (uncontrolled hypertension, cardiorespiratory impairment, cognition impairment with MMSE score less than 24, apraxia, severe hemi spatial neglect, musculoskeletal abnormalities, neurology impairment aside of stroke, visual impairment, hearing impairment, ataxia, severe sensory impairment, and involuntary movements) and had signed informed consent. The samples were chosen using consecutive sampling technique.

We found 38 subjects stroke patients that met the inclusion criteria and did not belong to exclusion criteria. We measured cardiorespiratory fitness using 6MWT and walking speed in Dr. Soetomo General Hospital Surabaya. Data analysis then was conducted using SPSS software. The study protocol was approved by the Ethics Committee of Dr. Soetomo General Hospital.

### Results

**Table 1. Walking speed measured using 10MWT (m/s)**

<table>
<thead>
<tr>
<th>10-mWT</th>
<th>N</th>
<th>Mean (m/dt)</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Self-speed</td>
<td>38</td>
<td>0.534 ±0.310m/s</td>
<td>0.085</td>
<td>1.127</td>
</tr>
<tr>
<td>Fast speed</td>
<td>38</td>
<td>0.629 ±0.385 m/s</td>
<td>0.083</td>
<td>1.3</td>
</tr>
</tbody>
</table>
Table 2. 10MWT assessment with self-speed and fast speed in male and female subjects, subjects with stroke onset of 4-48 weeks as well as unilateral and bilateral hemiparesis

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Self-speed (m/s)</th>
<th>Fast speed(m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Minimum</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>22</td>
<td>0.641±0.356</td>
<td>0.085</td>
</tr>
<tr>
<td>Female</td>
<td>16</td>
<td>0.389±0.141</td>
<td>0.094</td>
</tr>
<tr>
<td>Onset</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 &lt;24weeks</td>
<td>22</td>
<td>0.541±0.334</td>
<td>0.085</td>
</tr>
<tr>
<td>24-48 weeks</td>
<td>16</td>
<td>0.526±0.282</td>
<td>0.094</td>
</tr>
<tr>
<td>Paresis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unilateral hemi</td>
<td>32</td>
<td>0.577±0.315</td>
<td>0.085</td>
</tr>
<tr>
<td>Bilateral hemi</td>
<td>6</td>
<td>0.308±0.140</td>
<td>0.094</td>
</tr>
</tbody>
</table>

Table 3. Relation between 6MWT (VO2 max and mileage) and 10MWT (self-speed and fast speed)

<table>
<thead>
<tr>
<th>Variable</th>
<th>6MWT VO2 max (ml/kg/minute)</th>
<th>Mileage (m)</th>
<th>10-mWT (m/dt) Self-speed</th>
<th>Fast speed</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO2 max (ml/kg/minute)</td>
<td>r= 1.000 p= 0.000</td>
<td>r= 0.680 p= 0.000</td>
<td>r= 0.715 p= 0.000</td>
<td>r=0.687 p= 0.000</td>
</tr>
<tr>
<td>Mileage (m)</td>
<td>r= 0.680 p= 0.000</td>
<td>r= 1.000 p= 0.000</td>
<td>r= 0.756 p=0.000</td>
<td>r= 0.770 p=0.000</td>
</tr>
<tr>
<td>10-mWT Self-speed(m/s)</td>
<td>r=0.704 p=0.000</td>
<td>r= 0.756 p= 0.000</td>
<td>r= 1.000 p=0.000</td>
<td>r=.966 p=0.000</td>
</tr>
<tr>
<td>10-mWT Fast speed (m/s)</td>
<td>r=0.687 p=0.000</td>
<td>r=0.770 p=0.000</td>
<td>r=0.966 p=0.000</td>
<td>r= 1.000 p=0.000</td>
</tr>
</tbody>
</table>

*Annotation r = 0.6 medium correlation, r= >0.7 Strong correlation. Significant if (p= 0.05)

There were 32 subjects (84.21%) with unilateral hemiparesis and six subjects (15.79%) with bilateral hemiparesis. The number of subjects using walking aids was 20, while the other 18 subjects did not use walking aids.

Walking speed assessment in 38 subjects in this study using 10MWT indicated the results of mean time required to take 10 meters with self-speed was 0.534±0.0310 m/s. The shortest time to take 10 meters was 0.085 m/s and the longest time was 1.127 m/s. Walking speed with fast speed was 0.629±0.385 m/s with the shortest time of 0.083 m/s and the longest time of 0.083 m/s (Table 7). The mean of walking speed measurement results using 10MWT in 22 male subjects with self-speed was 0.641±0.356 m/s with minimum speed of 0.085 m/s and maximum speed of 1.127 m/s. As for fast speed, the mean was 0.769±0.442 m/s with
minimum speed of 0.083 m/s and maximum speed of 1.3 m/s. The mean of walking speed measurement results using 10MWT in 16 female subjects with self-speed was 0.389±0.141 m/s with minimum speed of 0.094 m/s and maximum speed of 0.599 m/s. As for fast speed, the mean was 0.438±0.160 m/s with minimum speed of 0.1 m/s and maximum speed of 0.710 m/s. The mean of walking speed measurement results using 10MWT in 22 subacute stroke patients with self-speed was 0.541±0.334 m/s with fastest speed of 0.085 m/s and the slowest speed of 1.127 m/s. As for fast speed, the mean was 0.635±0.431 m/s with the fastest speed of 0.083 m/s and the slowest speed of 1.493 m/s. The mean of walking speed measurement results using 10MWT in 22 chronic stroke patients with self-speed was 0.526±0.282 m/s with the fastest speed of 0.094 m/s and the slowest speed of 1.105 m/s. As for fast speed, the mean was 0.623±0.327 m/s with the fastest speed of 0.1 m/s and the slowest speed of 1.3 m/s.

The mean of walking speed measurement results using 10MWT in 32 hemiparesis unilateral stroke patients with self-speed was 0.577±0.315 m/s with the fastest speed of 0.85 m/s and the slowest speed of 1.170 m/s. As for fast speed, the mean was 0.690±0.389 m/s with fastest speed of 0.083 m/s and the slowest speed of 1.205 m/s. The mean of walking speed measurement results using 10MWT in six hemiparesis bilateral stroke patients with self-speed was 0.308±0.140 m/s with the fastest speed of 0.094 m/s and the slowest speed of 1.105 m/s. As for fast speed, the mean was 0.310±0.138 m/s with fastest speed of 0.1 m/s and the slowest speed of 1.493 m/s.

The relation between 6MWT (VO2max and mileage) and 10MWT (self-speed and fast speed)

The result of 6MWT measurement with mileage in meter indicated a strong correlation with 10MWT both self-speed ($r = 0.756 \ p = 0.000$) and fast speed ($r = 0.770 \ p = 0.000$). 6MWT measured with VOX2max (ml/kg/ minute) indicated strong correlation with 10MWT both with self-speed ($r = 0.715 \ p = 0.000$) and fast speed ($r = 0.687 \ p = 0.000$) (Table 9).

**Discussion**

The age of this study’s subjects vary between 30-65 years old with various Body Mass Index (BMI). Subjects were allowed to use walking aids during walking test.

Six-minute walk test was a simple, practical test that was easy to conduct, well-tolerated, and reflecting daily activity better than other walking tests. This test was conducted in a 10 feet (33m) hall. This test was designed to be an objective measurement for functional status and to represent cardiorespiratory fitness measurement in several individuals by converting the results of 6MWT walking test in VO2max. This test could also be used for measuring distances in meter taken by patients in six minutes. This test was able to make global and integrated evaluation from all involved system during practice including pulmonary and cardiovascular system, circulation system, peripheral circulation, blood, neuromuscular units and muscle metabolism.

Our study showed the same result in which we found VO2max decline in all samples, both subacute and chronic. This was in accordance with the result from Kelly et al. (2005 who reported cardiorespiratory fitness decline stroke onset of 4-7 weeks. Cardiorespiratory decline was significant physical impairment in post-stroke patients, regardless their genders, within approximately 30 days after stroke onset. Their findings also indicated that gait performance might be affected by cardiorespiratory fitness decline.

Our findings implied a relation between VO2max and mileage (Table 5.8). This corresponded with the statement from Kelly et al. (2005) that cardiorespiratory fitness measure was well-associated with gait performance where VO2max showed positive correlation with mileage in measurement using 6MWT in subacute stroke patients. Walking test was designed as an objective measurement for functional status and reflected cardiorespiratory measurement in several individuals. Correlation between 6MWT mileage (meter) and VO2max was within the range of 0.51-0.9.

Walking speed was a parameter of disability in walking fast and easily which was recommended as a output measuring instrument in stroke rehabilitation. Currently, there are several methods of walking speed measurement.

This study measured 10MWT walking speed in stroke patients with subacute and chronic onset where 20 subjects used walking aids and 18 others did not. The average of walking speed observation result in 22 male subjects with fast-speed was slower than with self-speed (see Table 8). This might occur when patients who had walking spasticity with fast speed would have stronger spasticity which inhibited patients in walking.
Calf muscle spasticity showed deviation of muscle tendon length pattern. The deviation pattern was more obvious in walking speed increase. In swing phase, spastic calf muscle was extracted one-third times faster than in normal state. The more deviation in muscle tendon length pattern led to the more rapid dependent velocity in spasticity. This inhibited patients’ walking process, especially in those with fast speed and therefore led to walking speed limitation in patients 11.

Observation result for correlation between 6MWT (by measuring mileage in meter) with 10MWT showed no significance (p=0.000) with correlation coefficient r=0.756. This is also in line with a study conducted by Scivoletto (2011) implying that both tests had inter and intra rater reliabilities and showed positive correlation with lower limb muscle strength 16.

The findings of this study suggesting a relation between upper and lower limb spasticity and 6MWT can be seen in Table 5.5 as stated by Ivey (2005). Mileage and walking speed were strongly related to spasticity. Skeletal muscle consisted of fibers expressing myosin heavy chain (MHC) isoform. MHC protein had higher oxidative function, resistant to fatigue and more sensitive for insulin-mediated glucose absorbance in paretic side thigh muscles. Fast and slow MHC fiber shift in thigh muscles occurred in post-stroke and it caused muscle fibers easier to get tired and there would be more insulin resistance. Low fast MHC fiber immunity led to dependency toward anaerobic or glycolytic energy production. In paretic side extremity, fast MHC fiber proportion showed strong negative relation with self-speed walking speed. This indicated that walking pattern neurological deficit walking pattern severity was correlated with 61% variants in fast MHC isoform 8. Aerobic capacity in individuals was correlated with cardiorespiratory fitness which was strongly associated with age, walking endurance and walking speed, and also associated with knee extensor muscle weakness during walking 17.

Cardiorespiratory fitness decline in post-subacute and chronic stroke patients caused difficulty in daily activities, particularly in walking. Walking was an indicator of independence in an individual. The findings in this study were expected to be able to increase awareness of the importance of cardiorespiratory fitness in daily activities and therefore it was important to conduct walking speed test and mileage in stroke patients that could independently walk or use walking aids.

The use of 6MWT and 10MWT was expected to be conducted with the proper standard and not only limited in patients in outpatient units, but also patients discharged from inpatient unit in order for us to be able to monitor patients’ fitness development objectively.

**Conclusion**

We found a relation between cardiorespiratory fitness and walking speed in post-subacute and chronic ischemic stroke.

**Conflict of Interest**: There is no conflict interest

**Source of Funding**: This study is self-funded

**Ethical of Clearance**: This study was approved by Ethical Commision of Health Research Faculty of Medicine University of Airlangga

**References**


Comparison Study of Urinary Retention Incidence in Assisted Vaginal Delivery Case with and without 24-Hour Catheterization

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2Head of Division of Urogynaecology and Reconstruction, Department of Obstetrics and Gynaecology, Faculty of Medicine, Universitas Indonesia - Dr. Cipto Mangunkusumo General Hospital, Jakarta 10430, Indonesia

Abstract

Background: Urinary retention is a condition commonly seen after vaginal delivery, especially in the high-risk cases, such as assisted vaginal delivery, grade 3-4th perineal rupture, or another high risk. Urinary retention caused by unsynchronized between the contraction of the bladder detrusor. Urinary catheterization is one of the preventions of urinary retention. It gives a time for perineal trauma to relieve and no longer edema, so that urethra can be fully relaxed. Therefore, 24-hour catheterization expected to prevent bladder overdistention. It also prevents the bladder from becoming atonia.

Objective: This research aims to study the incidence of urinary retention in assisted vaginal delivery with and without 24-hour catheterization.

Method: This study used randomized control trials that compared two groups with 24-hour catheterization and without 24-hour catheterization. This study was conducted on 40 women in each group who experienced assisted vaginal birth at Dr Soetomo Hospital, Indonesia.

Result: Six women (15%) experienced urinary retention with 24-hour catheterization and six women (15%) had urinary retention without catheterization. There was no significant difference in the incidence of urinary retention with 24-hour catheterization and without 24-hour catheterization (p-value = 1.00). Also, there was no significant interference of urinary retention in the normal and prolonged second stage of labor (p-value = 0.736), and there was no significant risk factor contributing to urinary retention.

Conclusion: No significant difference in urinary retention occurred in assisted vaginal delivery with and without 24-hour catheterization.

Keywords: urinary retention, assisted vaginal delivery, catheterization.

Introduction

Urinary retention is a condition commonly seen in post vaginal delivery1-3. This condition may become bladder overdistention and atrophy of bladder detrusor. If not treated immediately, this condition may become worse and increase into hospital stay 4,5. If not treated properly, urinary retention will turn into acute urinary retention 6,7. The variation incidence may occur due
to the broad definition of urinary retention. Urinary retention may be classified as overt and covert. Overt type is symptomatic, characterized by the inability to spontaneous micturition wheater covert is a type of asymptomatic while the patient cannot empty the bladder adequately so there is a little residual volume in the bladder. This classification often difficult to classified due to the difference in residual urine volume between 50-250 ml. Urinary retention incidence also varied in some country. In Canada, according to Musselwhite, urinary retention incidence occurs in 4.7%. In Denmark, previous research report it only 0.7% in 2003. Lim KJ report 6% case from 860 vaginal delivery in Korea. Glavind K and Bjork J report that 33% of women with assisted vaginal delivery experience urinary retention. Also, 17% of episiotomy may complicate urinary retention and 33% of anal sphincter rupture contribute and 33% use of epidural anesthesia may contribute as well to urinary retention. A significant risk contributed to assisted vaginal delivery and sphincter rupture. In 2010, previous study reported 10.6% urinary retention case in India. An assisted vaginal delivery and longer stage of labor significantly contribute to urinary retention. Primiparity, epidural anesthesia, assisted vaginal delivery, episiotomy, prolonged stage 1, and stage 2 labor significantly increase the risk of urinary retention. In 35 women who underwent assisted vaginal delivery, 16 of them experience urinary retention. Carley stated that assisted vaginal surgery and epidural anesthesia are independent risk factors. Yip stated urinary retention after labor may worry about the impact, even after 2-5 days of recovery.

Unidentified urinary retention may over distended bladder and need prolonged catheter use. The use of a 24-hour catheter in post vaginal delivery women with the risk factors of urinary retention is expected to prevent overdistention of the bladder. Catheters are also often used for Ventriculo Peritoneal (VP) bypass surgery, and the resulting complications are very rare. At Dr Soetomo Hospital, Surabaya, there is no certain protocol to prevent urinary retention, including 24-hour catheterization in post vaginal delivery. Since assisted vaginal delivery significantly risks to cause urinary retention, this study aims to compare the incidence of urinary retention in assisted vaginal delivery with and without 24-hour catheterization.

Method

This randomized control trial study was conducted at Dr. Soetomo General Hospital Surabaya for nine months. All subjects were gathered by consecutive sampling. The study population divided into two groups with a random lottery. One group consisted of women who underwent assisted vaginal surgery, then were treated with 24-hour catheterization, the other group was without 24-hour catheterization. Each group used a female catheter no.12 to gather residual urine and to ensure there was no residual urine bladder. Analgesia with sodium diclofenac was administered to women with post assisted vaginal delivery.

In the group with 24-hour catheterization after 24 hours, the catheter use was discontinued then observed for 6 hours. The residual urine samples were collected and classified in urinary retention if the residual urine volume were 200 ml or more. The group without 24-hour catheterization was observed for 6 hours after labor. If a subject could not spontaneously urinate in 6 hours, She was then treated the same as the 24-hour catheterization group. The residual urine volume was calculated by ultrasonography using the Koelbl formula described in Figure 1. The statistical Analysis employed T unpaired test, Chi-square test, and the Mann-Whitney test. All analyses were conducted using SPSS.

Results

As 80 subjects met the inclusion criterion, all subjects had homogenous characteristics in age, newborn birth weight, parity, instrumentation during labor, episiotomy, and perineal rupture. In stage 2 labor. As many as 75% of the women in the control group and 50% of the women in the experimental group had a prolonged second stage. These women had experienced assisted vaginal surgery before. A chi-square test was used to compare both groups with significant test results (p-value = 0.021).

The urinary retention was classified if the residual urine volume was more than 200 ml. Statistical analysis using chi-square revealed that there were no significant differences between the two groups (p-value = 1.00), as displayed in Table 2. To identify whether there was the interference of the second stage of labor in urinary retention, a statistical analysis using the Fischer test was performed. The analysis revealed no significant difference between the two groups as shown in Table 3. These subject risk factors were present and could affect
the incidence of urinary retention. To identify whether there was an interference of risk factors, multivariate analysis was conducted as displayed in Table 4. The analysis revealed no significant risk factor contributing to urinary retention (p-value >0.05).

Figure 1. Koelbl formula, \( V = 0.7 \times H \times W \times DL \). \( H = \text{height} \), highest superoinferior diameter, \( DL = \text{depth} \), highest anteroposterior diameter, the height and depth are measured from sagital aspect. \( W = \text{width} \), is the highest anterolateral diameter measured from the transversal aspect. \( KK = \text{bladder} \).

Table 1. Subject Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Without 24-hour catheterization (n=40)</th>
<th>With 24-hour catheterization (n=40)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Mean + sd)</td>
<td>27.42 ± 6.06</td>
<td>28.85 ± 6.51</td>
<td>0.314*</td>
</tr>
<tr>
<td>Birth weight (gram)</td>
<td>3177 ± 361</td>
<td>3105 ± 452</td>
<td>0.431*</td>
</tr>
<tr>
<td>Second stage of labor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prolonged</td>
<td>30 (75%)</td>
<td>20 (50%)</td>
<td>0.021**</td>
</tr>
<tr>
<td>Normal</td>
<td>10 (25%)</td>
<td>20 (50%)</td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primiparity</td>
<td>20 (50%)</td>
<td>21 (52.5%)</td>
<td>0.823**</td>
</tr>
<tr>
<td>Multiparity</td>
<td>20 (50%)</td>
<td>19 (47.5%)</td>
<td></td>
</tr>
<tr>
<td>Instrumentation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaccum</td>
<td>37 (92.5%)</td>
<td>31 (77.5%)</td>
<td>0.060**</td>
</tr>
<tr>
<td>Forceps</td>
<td>3 (7.5%)</td>
<td>9 (22.5%)</td>
<td></td>
</tr>
<tr>
<td>Episiotomy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>37 (92.5%)</td>
<td>37 (92.5%)</td>
<td>1.000***</td>
</tr>
<tr>
<td>No</td>
<td>3 (7.5%)</td>
<td>3 (7.5%)</td>
<td></td>
</tr>
<tr>
<td>Perineal rupture</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>2 (5.0%)</td>
<td>0 (0%)</td>
<td>0.198***</td>
</tr>
<tr>
<td>Grade 1-2</td>
<td>14 (35.0%)</td>
<td>23 (57.5%)</td>
<td></td>
</tr>
<tr>
<td>Grade 3-4</td>
<td>24 (60%)</td>
<td>17 (42.5%)</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Incidence of urinary retention between two groups

<table>
<thead>
<tr>
<th>Urinary retention</th>
<th>Without 24-hour catheterization</th>
<th>With 24-hour catheterization</th>
<th>Total</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>6 (15%)</td>
<td>6 (15%)</td>
<td>12 (15%)</td>
<td>1.000</td>
</tr>
<tr>
<td>No</td>
<td>34 (85%)</td>
<td>34 (85%)</td>
<td>68 (85%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>40 (100%)</td>
<td>40 (100%)</td>
<td>80 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Interference of the second stage of labor in urinary retention

<table>
<thead>
<tr>
<th>Stage 2 of labor</th>
<th>Urinary retention (-)</th>
<th>Urinary retention (+)</th>
<th>Total</th>
<th>P-value (Fischer test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prolonged duration</td>
<td>Catheter(-)</td>
<td>24 (80%)</td>
<td>6 (20%)</td>
<td>30 (100%)</td>
</tr>
<tr>
<td></td>
<td>Catheter(+)</td>
<td>15 (75%)</td>
<td>5 (25%)</td>
<td>20 (100%)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>39 (78%)</td>
<td>11 (22%)</td>
<td>50 (100%)</td>
</tr>
<tr>
<td>Normal duration</td>
<td>Catheter (-)</td>
<td>10 (100%)</td>
<td>0 (0%)</td>
<td>10 (100%)</td>
</tr>
<tr>
<td></td>
<td>Catheter (+)</td>
<td>19 (95%)</td>
<td>1 (5%)</td>
<td>20 (100%)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>29 (96.7%)</td>
<td>1 (3.3%)</td>
<td>30 (100%)</td>
</tr>
</tbody>
</table>

Table 4. Interference of subject risk factor in urinary retention.

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>Odd ratio</th>
<th>P value</th>
<th>CI 95% (min-maks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.007</td>
<td>0.99</td>
<td>0.894</td>
<td>0.90-1.10</td>
</tr>
<tr>
<td>Birth weight</td>
<td>0.001</td>
<td>1.001</td>
<td>0.194</td>
<td>0.999-1.003</td>
</tr>
<tr>
<td>Second stage of labor</td>
<td>2.102</td>
<td>8.179</td>
<td>0.05</td>
<td>0.999-66.980</td>
</tr>
<tr>
<td>Parity</td>
<td>0.870</td>
<td>2.387</td>
<td>0.187</td>
<td>0.656-8.686</td>
</tr>
<tr>
<td>Episiotomy</td>
<td>1.163</td>
<td>3.200</td>
<td>0.211</td>
<td>0.517-19.820</td>
</tr>
<tr>
<td>Perineal rupture</td>
<td>-0.121</td>
<td>0.886</td>
<td>0.847</td>
<td>0.259-3.032</td>
</tr>
</tbody>
</table>

Discussion

These complex factors explained why there is no significant difference in urinary retention incidence between both groups. Therefore, 24-hour catheterization is expected to prevent bladder overdistention. It also prevents the bladder from becoming atonia. Traumatic factors such as the vulva or urethral injury cannot be eliminated. Nevertheless, the micturition requires coordination between detrusor muscle contraction and urethral relaxation. Subject characteristics in this study become known risk factors of urinary retention. Many studies report consistent risk factors for urinary retention. A previous study of a case-control study reported 52 cases of overt urinary retention in 860 postpartum women. Risk factors, such as perineal trauma and prolonged second stage, are significant in urinary retention.
In this study at Dr. Soetomo General Hospital, mediolateral episiotomy also contributed to risk factors compared to the subjects who do not undergo episiotomy (OR 3.2). Some study reported that assisted vaginal surgery is a significant risk factor for urinary retention. In this study, the researchers utilized 24-hour catheterization to prevent the weakening of detrusor muscle due to overdistention. In the group without 24-hour catheterization, a researcher found six subjects with urinary retention due to the inability to urinate in the first six hours or residual urine more than 200 ml, and 34 subjects could spontaneously urinate in six hours.

In the group with 24-hour catheterization, six subjects had urinary retention. One subject had symptom relief after first try intermittent catheterization, and five subjects were relieved after two times intermittent catheterization. Statistical analysis, t unpaired test revealed no significant difference between both groups. In this study, the second stage of labor was found as a confounding factor. In the group without 24-hour catheterization, the subjects with the prolonged second stage were greater than in the group with 24-hour catheterization. This factor was significant. To eliminate this factor, statistical analysis was conducted using the Fischer test. The result was there no significant interference of urinary retention in the group with 24-hour catheterization, where the subjects experienced a prolonged second stage or normal second stage of labor.

According to the previous study, urinary retention had occurred due to the neurological dysfunction, immobilization, trauma of the vulva, abnormality in bladder contraction, spasm of the external urethra due to perineal rupture. In this study, all subjects were administered with mefenamic acid as analgesia. Risk factors such as the duration of the second stage of labor (OR 8.179), parity (2.387), and episiotomy procedure (3.200) contributed to urinary retention incidence, respectively.

Conclusion

There is no significant difference in urinary retention incidence in assisted vaginal delivery with and without 24-hour catheterization. However, this study requires more research with a bigger number of samples to determine the effect of 24-hour catheterization as prevention of urinary retention in assisted vaginal surgery.

**Conflict of Interest:** There is no conflict of interest

**Source of Funding:** All of the cost and fees related with this research are paid by the authors only with no sponsorship nor external funds.

**Ethical Clearance:** This research involves participants in the process using a questionnaire that was accordant with the ethical research principle based on the regulation of research ethic regulation. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, non-maleficence, and justice.

**References**


6. Kurniasari D., Budiono, Tarmono, Hardjowijoto S. Predicts the successfulness of a trial voiding without catheter (Twoc) through urine retention volume, detrusor wall thickness (dwt) and intravesical protrusion of prostate (ipp) on acute


Effectiveness of Additional Intranasal Phototherapy through Decrease Score of Nose Symptom and Eosinophil Mucosa on Persistent Allergic Rhinitis

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Abstract

Background: Allergic rhinitis (AR) is a common atopic disease but the available treatment has limited use and success rate. It has been suggested that intranasal phototherapy represents an alternative choice in the treatment of AR.

Objective: This study aimed to analyze the effect of additional intranasal phototherapy to loratadine therapy compared with single loratadine therapy in order to decrease total nasal symptoms score (TNSS) and nasal mucosa eosinophil count in persistent AR patients.

Method: Fifty-four persistent AR patients were divided into two treatment groups: group A and B treated with 10 mg loratadine once a day with additional intranasal phototherapy at 660 nm. 4.4 minutes 3 times a day for group B. Evaluation was based on TNSS and nasal mucosa eosinophil count pre and post 14 days of treatment.

Result: The study conducted from July to August 2014 demonstrated significant average of TNSS decrease on group B compared to group A (p = 0.002). Also significant average of eosinophil mucosa nasal count decrease on group B compared to group A (p = 0.049). Both group A and B showed the smallest score of TNSS pre-therapy was 4 vs 6, however the highest score was 6 vs 12. Group A and B showed the smallest score of TNSS post-therapy was 0 vs 0, however the highest score was 8 vs 7.

Conclusion: Additional intranasal phototherapy to loratadine therapy showed more effective compared with single loratadine therapy in order to decrease TNSS and nasal mucosa eosinophil count in persistent AR patients.

Keywords: Allergic rhinitis, Eosinophil, Phototherapy intranasal, Loratadine.

Introduction

Allergic rhinitis (AR) is the most common atopic disease that becoming global health problem. In persistent AR, the symptoms happen more than 4 weeks and characterized by the accumulation of inflammatory cells, especially eosinophils. Clinical evaluation and therapy of persistent AR can be performed subjectively by calculating the total nasal symptom score (TNSS) and objectively on nasal mucosal eosinophil1. Histamine interacts with H1 and H2 receptors increased permeability of blood vessels causes nasal congestion2,3. Histamine induces the expression of intracellular adhesion molecule-1 (ICAM-1) to help eosinophils migration to the nasal mucosa4. Eosinophils actively secrete chemical mediators and Reactive oxygen species (ROS) that worsen AR symptoms5.

First-line therapy recommended Allergic rhinitis is loratadine, which can be administered singly or in combination with other anti-allergic drugs6,7. Loratadine inhibits eosinophils adhesion in the blood

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vessels and eosinophils accumulation in nasal mucosa. Significant decrease of eosinophils infiltration, the levels of eosinophil cationic protein (ECP), histamine and expression of ICAM-1 on RA patient’s nasal rinse. Therefore some experts develop new therapeutic modalities of intranasal phototherapy to optimalize AR therapy.

Intranasal phototherapy with visible red light inhibits inflammatory mediators release by eosinophils and stimulate nitric oxide (NO). Nitric oxide inhibits mast cell degranulation and cytokine expression. Increased vascular permeability as well as adhesion and leukocyte migration ultimately suppress the symptoms of AR. An intranasal phototherapy study with visible red light 660±5 nm wavelength showed improvement in AR clinical symptoms of 70%. Intranasal phototherapy with a combination of UVA, UVB and visible light (mUV/vis) significantly decreased TNSS as well as the number inflammatory cells and mediators and were conducted with the aim of comparing the effectiveness of mUV/vis intranasal phototherapy with AH fexofenadine hydrochloride.

Separate research on the effectiveness of loratadine and intranasal phototherapy has been widely practiced abroad. Up to now, there has been no research on the effectiveness of intranasal phototherapy addition to loratadine therapy. Based on the above description the researchers aimed to determine the effect of intranasal phototherapy addition to loratadine therapy compared to single loratadine therapy in persistent AR patients which was assessed by TNSS and the number of nasal mucosal eosinophils.

**Method**

This study used a single-blind randomized controlled trial design with pre and post-test design which was held in January to November 2014 at the Outpatient Unit of Allergy Immunology Division and Clinical Pathology Installation of Dr. Soetomo Teaching Hospital, Surabaya. The samples were divided into 2 groups by block randomization with the number of 4 blocks to obtained 6 permutations. The control group (group B) received loratadine therapy and the study group (group A) received loratadine therapy combined with intranasal phototherapy. The study sample was mild to moderate-severe AR patients according to ARIA criterion 2008. All samples underwent TNSS and nasal mucosa eosinophil pre- and post-therapy.

This study used consecutive sampling with inclusive and exclusive criteria. The inclusion criteria were having a pre-therapy GHT score ≥4, aged between 21 and 60 years, free anti-allergic drugs such as: AH (1 week), systemic corticosteroids (4 weeks), topical corticosteroids (2 weeks), topical decongestants (1 week), anticholinergic and chromoline (for 2 weeks), willing to take part in the research and signing the approval sheet to follow the research. The exclusion criteria were the presence of acute respiratory infections, acute and chronic paranasal sinusitis, medical rhinitis, nose abnormalities such as tumors, nasal polyp, severe deviation septum in conditions of pregnancy or lactation and having a history of loratadine allergy.

This research required 10 mg loratadine tablets and 8 units of intranasal phototherapy devices. Intranasal phototherapy equipment used was Bionase unit of Syrolight. Bionase unit consisted of control box size 23 x 16 x 6 cm, 246 grams and 2 LED probes (Israel). The nasal mucosal eosinophil was applied to the glass object and then dried. Those preparations were stained with Wright staining and then examined their eosinophils using Olympus microscope with 1000 magnification. The sample was not allowed to receive other treatments that could interfere with the results of the study for 14 days. Then TNSS and nasal mucosal eosinophil were assessed.

The data were tabulated and analysed statistically using SPSS (SPSS. Inc. Chicago, IL) with significance level (p) of 0.05 or 5%. Analysis of age distribution, TNSS, and number of nasal mucosal eosinophils were obtained with Mann-Whitney U test. Sex distribution was analysed using Chi square test and side effects was with Fisher’s exact test. The Wilcoxon signed rank test was used to determine the ratio of TNSS and the number of nasal mucosal post eosinophils between the two groups.

**Results**

**TNSS score and Therapy**

Group A had the smallest TNSS pre-therapy of 4 and the highest score of 11 (1 patient each, 3.7%). Group B had the smallest TNSS pre-therapy of 6 (3 patients, 11.1%) and the highest score of 12 (2 patients, 2.4%). Group A had the lowest TNSS post-therapy of 0 (3 patients, 11.1%) and group B had the lowest TNSS post-
therapy of 0 (1 patient, 3.7%). Group A had the highest TNSS post-therapy of 8 (1 patient, 3.7%) while group B was 6 patients (3.7%). The average TNSS pre-therapy in group A was 7.67 (1.73) and group B was 9.41 (1.80). The average TNSS post-therapy in group A was 3.63 (2.08) and group B was 78 (1.40, Table 1).

The average percentage of TNSS decrease post-therapy in group A was 55.2% while group B was 70.9%. The Independent sample t-test showed that the percentage between group A and B was significantly different (p = 0.002, Table 2).

**Nasal Mucosa Eosinophil and Therapy**

Groups A and B had the most eosinophils pre-therapy in the range of ≥10% to <25%, respectively. Group A had the least eosinophils pre-therapy in the range of ≥25% to <50% while group B was <10%. The average eosinophil pre-therapy in group A was 21.44 (19.14) and B was 32.93 (26.46).

Group A had the most eosinophils post-therapy of <10% and ≥10% to <25%. Group B had the most eosinophils post therapy of <10%. Group A had the least eosinophils post therapy of ≥50% while group B was ≥25% - <50% and ≥50%. The average eosinophils post-therapy in group A was 12.48 (14.93) and group B was 8.78 (12.75, Table 3.)

The average percentage of nasal mucosa eosinophil decrease in group A was 22.6% and group B was 64.5%. The independent sample t-test showed that the percentage decrease of nasal mucosal eosinophils between group A and group B was significantly different (p = 0.049, Table 4.)

**Side Effect**

The most common side effects reported by patients in groups A and B were drowsy. Dry mouth or throat in both groups happened in 1 patient (3.7%). The Fisher’s exact test suggested that the distribution of side effect between the two groups was not significantly different (p = 1.000, Table 5).

**Table 1. Total Nasal Symptoms Score Pre and Post Therapy**

<table>
<thead>
<tr>
<th>TNSS</th>
<th>Pre-Therapy (%)</th>
<th>Post-Therapy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A</td>
<td>Group B</td>
</tr>
<tr>
<td>0</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>1</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>2</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>3</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>4</td>
<td>1 (3.7)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>5</td>
<td>1 (3.7)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>6</td>
<td>5 (18.5)</td>
<td>3 (11.1)</td>
</tr>
<tr>
<td>7</td>
<td>6 (22.2)</td>
<td>1 (3.7)</td>
</tr>
<tr>
<td>8</td>
<td>7 (25.9)</td>
<td>5 (18.5)</td>
</tr>
<tr>
<td>9</td>
<td>1 (3.7)</td>
<td>2 (7.4)</td>
</tr>
<tr>
<td>10</td>
<td>5 (18.5)</td>
<td>7 (25.9)</td>
</tr>
<tr>
<td>11</td>
<td>1 (3.7)</td>
<td>7 (25.9)</td>
</tr>
<tr>
<td>12</td>
<td>0 (0.0)</td>
<td>2 (7.4)</td>
</tr>
<tr>
<td>Average (SD)</td>
<td>7.67 (1.73)</td>
<td>9.41 (1.80)</td>
</tr>
<tr>
<td>Total (%)</td>
<td>27 (100)</td>
<td>27 (100)</td>
</tr>
</tbody>
</table>
### Table 2. Average Percentage of Total Nasal Symptoms Score Decrease Pre and Post-therapy

<table>
<thead>
<tr>
<th>TNSS Decrease</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>Average (%)</td>
<td>55.2</td>
<td>70.9</td>
</tr>
<tr>
<td>Standard Deviation (%)</td>
<td>21.6</td>
<td>12.7</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>0.002</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3. Total Nasal Symptoms Score Pre and Post-therapy

<table>
<thead>
<tr>
<th>Eosinophil (%)</th>
<th>Pre-therapy</th>
<th>Post-therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group A</td>
<td>Group B</td>
</tr>
<tr>
<td>&lt;10</td>
<td>5 (18.5)</td>
<td>3 (11.1)</td>
</tr>
<tr>
<td>≥10–&lt;25</td>
<td>15 (55.6)</td>
<td>11 (40.7)</td>
</tr>
<tr>
<td>≥ 25–&lt;50</td>
<td>3 (11.1)</td>
<td>6 (22.2)</td>
</tr>
<tr>
<td>≥50</td>
<td>4 (14.8)</td>
<td>7 (25.9)</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>100</td>
</tr>
</tbody>
</table>

### Table 4. Average Percentage of Nasal Mucosa Eosinophil Decrease

<table>
<thead>
<tr>
<th>Eosinophil Decrease</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>Average (%)</td>
<td>22.6</td>
<td>64.5</td>
</tr>
<tr>
<td>Standard Deviation (%)</td>
<td>88.4</td>
<td>52.5</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>0.049</td>
<td></td>
</tr>
</tbody>
</table>

### Table 5. Side Effect

<table>
<thead>
<tr>
<th>Side Effect</th>
<th>Group A N (%)</th>
<th>Group B N (%)</th>
<th>Total (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drowsy</td>
<td>5 (18.5)</td>
<td>5 (18.5)</td>
<td>10 (18.5)</td>
<td>1.000</td>
</tr>
<tr>
<td>Dry Nasal</td>
<td>0 (0.0%)</td>
<td>1 (3.7)</td>
<td>1 (1.9)</td>
<td></td>
</tr>
<tr>
<td>Dry Mouth/throat</td>
<td>1 (3.7)</td>
<td>1 (3.7)</td>
<td>2 (3.7)</td>
<td></td>
</tr>
<tr>
<td>Dizzy</td>
<td>1 (3.7)</td>
<td>0 (0.0)</td>
<td>1 (1.9)</td>
<td></td>
</tr>
<tr>
<td>No symptom</td>
<td>20 (74.1)</td>
<td>20 (74.1)</td>
<td>40 (74.1)</td>
<td></td>
</tr>
<tr>
<td>Total (%)</td>
<td>27 (100)</td>
<td>27 (100)</td>
<td>54 (100)</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

The results showed significant improvement of nasal congestion and rhinorrhea 80% and 81% (study group) and 31% and 14% (control group) respectively. A study investigated the effects of mUV/vis intranasal phototherapy versus topical azelastine therapy in moderate persistent AR. The average variation of TNSS pre and post-therapy might be due to the different sample criteria selected. This leads to differences in average TNSS pre-therapy as well as the possibility of decreasing TNSS as being summarized as average TNSS post-therapy. Another study using Bionase phototherapy tool compared 660 nm visible red light effect (study group) and sham illumination (control group). A similar study reported a decrease of all AR symptoms post 660 nm visible red light phototherapy. Other studies showed a decrease in TNSS after intranasal phototherapy of visible red light for 14 days.

Other studies used different light showing a significant improvement in TNSS post-mUV/vis phototherapy of 50% in 11 patients (61.1%). The Hex fexofenadine group showed no significant improvement with 50% TNSS improvement in 2 patients (15.4%). This study used the Bionase intranasal phototherapy apparatus with LLLT technique did not cause any harmful side effects or interactions with. Bionase efficiency as AR therapy occurred because of its role in producing NO. In this study the number of nasal mucosal eosinophil that could be analyzed was 24 of 27 patients in group A and 25 of 27 patients in group B. Three preparations of group A and two preparations of group B were not included in the statistical analysis.

Missing 5 samples happened because it had a pre value of 0 therefore in statistical analysis the result was not defined. Other descriptive research data on average percentage of nasal mucosal eosinophil pre- and post-loratadine with and without visible red light phototherapy were not found. The average percentage of nasal mucosal eosinophil decrease between the two groups was significantly different (p = 0.049). The average of nasal mucosal eosinophil decrease in group A was 22.63% smaller than the decrease in group B that was 64.46% although sample size for percentage analysis was below the minimum but with significant analysis results, the lack of eosinophil samples did not become obstacles and the results of the analysis were still acceptable.

A study showed similar results in decreasing the inflammatory cells (eosinophils, macrophages, neutrophils, lymphocytes) post-visible red light. Previous studies showed a significant decrease of eosinophils, ECP and IL-5 post- mUV/vis intranasal phototherapy and visible red light. Other studies showed a significant decrease in eosinophils, inflammatory cells, ECP, histamine and ICAM-1 expression after loratadine therapy. Eosinophils reached nasal secretions in nasal cavity within 1 - 3 hours and stayed up to 3 days. In this study Bionase phototherapy worked by producing NO which would inhibit eosinophil cell adhesion. NO also played a direct role in modulation of immune response by influencing expression of adhesion molecule VCAM-1, ICAM-1 and endothelial E-selectin that were important for eosinophil adhesion. Visible red light phototherapy with LLLT technique significantly interfered major transcription factors activity that governed IL-4 expression, IL-5 and eotaxin thus it could inhibited IgE production and eosinophil recruitment. Research conducted on mice’s BALF showed decreased infiltration of inflammatory cells (eosinophils, macrophages, neutrophils, lymphocytes), IL-4, IgE secretion and increased IFN-γ after phototherapy. The effects of phototherapy were reported having no side effects. Side effects between two groups were not significantly different. Another study obtained dry nose, mouth and throat complaints post-loratadine in 23% of patients.

Conclusion

The addition of intranasal phototherapy to loratadine therapy compared to loratadine therapy singly was more effective in reducing TNSS and the number of nasal mucosal eosinophils of AR patients.

Ethical Clearance: The study protocol has been approved by the Medical Research Ethics Commission of Dr. Soetomo Teaching Hospital.

Source of Funding: This research using personal funding. There is no organization or university that give some funding in this research.

Conflict of Interest: There is no conflict of interest.

References


Hydration Status and Degree of Clinical Severity Patients with Thrombotic Infarction Stroke

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Abstract

Background: Dehydration is common in acute ischemic stroke patients and associated with a poor increase in clinical outcomes. It is caused of the increase of hematocrit related to the infarct volume which is wider to the cerebra infarct patients. It is also related to the repeatedly emboli stroke and thromboembolic vena after the occurrence of the acute stroke.

Objectives: To analyze the correlation between hydration status and clinical severity in patients with acute thrombotic stroke.

Methods: This study was conducted by using control design consecutive admission sampling technique. Serum BUN and creatinine measurements were performed when patients were administered to the hospital. Dehydration was defined if the ratio of BUN/serum creatinine was >15. Patients were divided into dehydration and non-dehydrated groups. The degree of clinical severity was measured by using the National Institute of Health Stroke Scale (NIHSS) at 7 days of onset of stroke. Data were analyzed using chi square, fisher, t dependent test, and mann whitney (p <0.05).

Result: There were 19.23% stroke patients with dehydration were in the case group and the control group were 9.62%. There was no significant correlation between hydration status and clinical severity in acute thrombotic stroke patients (p = 0.126; OR = 2.625).

Conclusion: There was no correlation between hydration status and clinical severity in acute thrombotic stroke.

Keywords: Stroke, dehydration status, BUN/SK, NIHSS

Introduction

Stroke is the first leading cause of disability worldwide, the second most common cause of dementia, and the third leading cause of death. Stroke resulted in major clinical, social, and economic impacts with a total death rate of 6.2 million (+11% of the total). Around 9 million people suffered a stroke in 2008, 30 million people have had previous strokes and still alive. Stroke is the main cause of disability and this recent decline has fallen from the third leading cause of death to the fourth place in the United States ¹. The incidence of stroke has increased exponentially over the last 30 years and its causes vary by age. Ninety-five percent of strokes occur in people over the age of 45 and two-thirds of strokes occur in people over age 65. The risk of death in a person with stroke also increases with age. Stroke can occur at any age including the age of the children ².

Stroke is also a major cause of disability. It is approximately 55% of patients who survive up to 3 years will experience a decrease in quality of life. There is only 20% of patients with stroke can return to work as before ³,4. The association between renal function and stroke has been determined by a study assessing the association between hypertension and mortality with black patients suffering from acute stroke as the determinants. Stroke survivors have lower levels of...
urea than those who died. Friedman’s findings of stroke survivors in New Zealand mentioned that serum creatinine concentrations independently as predictors of mortality even after adjustment for confounding variables.

Euvolemia is important for adequate blood flow and maximizing oxygen intake into optimal tissue and organ function. Research showed blood flow to organs such as muscles and kidneys decreases significantly during dehydration. Cardiac output also decreases during dehydration. The brain has the ability to control blood flow through autoregulation that regulated constant cerebral perfusion pressure even when blood pressure changes. Dehydration has been shown to decrease the autoregulation response to orthostatic position changes, but in general does not decrease cerebral perfusion.

The role of dehydration in cerebral infarcts is thought to be multifactorial. Dehydration increases the viscosity of blood by decreasing blood flow to the brain through the decrease of intravascular volume. The increase hematocrit has been shown to be associated by a larger volume of infarction in patients with cerebral infarction. Dehydration has also been shown to be associated by recurrent embolic strokes and thrombotic events including venous thromboembolism following the onset of acute stroke. Dehydration can disrupt the distribution of oxygen to the brain, increase the risk of venous thromboembolism, and be associated with poor outcome in stroke. Dehydration can be detected by biomarkers of reduced fluid in the blood. Biomarkers that are often used to assess the presence of dehydration are the ratio of BUN/creatinine and plasma osmolality.

There is no hydration marker has been considered a golden standard, especially for mild dehydration. A total of 102 elderly patients were admitted to one of the educational hospitals who were diagnosed dehydrated. After a serum analysis, there is only 17% had an increase in serum osmolarity, 11% had elevated serum sodium, and 68% had an increase in blood urea nitrogen (BUN)/serum creatinine (SCr). BUN and SC are valuable laboratory parameters for evaluating renal function. The ratio of BUN to serum creatinine (BUN/SCr) is one of the useful laboratory indicators to determine the decrease in hydration status. Based on the description above, it is necessary to conduct an analysis of dehydration status and degree of clinical severity in patients with thrombolytic infarction.

**Methods**

The participants in this study were all acute thrombotic stroke patients who met the inclusion and exclusion criteria. The inclusion criteria included patients diagnosed clinical acute thrombotic stroke and uncompromised CT scan, first stroke occurring within 24 hours, and having National Institute of Health Stroke Scale (NIHSS) score score of <4. NIHSS is a tool used to assess stroke damage with score range of 0 to 42. Exclusion criteria include patients having chronic renal failure, congestive heart failure, hemorrhagic stroke, embolism stroke, systemic inflammatory response syndrome, and use diuretic drugs. Participants who were willing to be involved in this study were required to fill the informed consent form in advance.

The participants were selected by identifying the number of patients with acute thrombolytic stroke in the previous year at Dr. Soetomo Teaching Hospital Surabaya, Indonesia. Afterwards, we found 101 participants with acute thrombolytic stroke who met the inclusion and exclusion criteria. During the course of the study, some patients with acute thrombolytic stroke were excluded from the study because the participant went home forcibly or died in ≤24 hours before blood sampling was taken for BUN and SCr examination. After those processes, we found 52 participants that were able to be enrolled in this study.

The procedure of this study involved patients diagnosed with acute thrombolytic stroke. Patients were examined by NIHSS on the seventh day. Participants were identified and divided into 2 groups, i.e. case group (moderate NIHSS with score 4-15) and control group (NIHSS with score <4). We conducted blood pressure measurement for the participants. Blood sample of 3 ml was stored in EDTA vials and identified for the BUN, SCr, Hb, Albumin, Potassium, Sodium, and Glucose. Assessment of dehydration status (BUN/SCr) was based on two studies. The criteria of hypertension refers to Joint National Committee 8 (JNC-8).

The measurement data were analyzed according to the type of measurement results data using SPSS 22.0 (SPSS, Inc., Chicago, IL). Analysis on the participants’ gender data and blood pressure were conducted using chi square test (A p value of <0.05 was considered as
statistically significant). Analysis on age, albumin, sodium, potassium, hemoglobin, and glucose data of the participants were conducted using t-dependent test (A p value of <0.05 was considered as statistically significant) with Kolmogorov Smirnov beforehand. The correlation between dehydration status and degree of clinical severity in stroke infarction patients was analyzed using chi square test (A p value of <0.05 was considered as statistically significant).

Result

Sample Characteristic

The demographic data in this study consisted of two types of data, i.e. gender and age. In case group, most participants were female (32.69%), while in control group most participants were male (32.69%). The comparison of age between the two groups showed p = 0.027 (Table 2). In case group, the mean of the participants’ age was 55.43±9.05 years old, while in control group was 53.12±11.96 years old. The comparison of age between the two groups showed p = 0.233 (Table 1).

The Albumin, Glucose, Potassium and Sodium Level

The clinical data of albumin examination showed the value 4.00±0.36 mg/dl in case group and 4.18±0.42 mg/dl in control group. The comparison between the two groups showed p = 0.276. The sodium level was 134.12±3.38 mg/dL in case group and 135.12±2.52 mg/dL in control group. The comparison of sodium level between the two groups showed p = 0.653. The potassium level in case group was 3.76±0.53 mg/dL, while in control group was 4.05±0.46 mg/dL. The comparison of potassium level between the two groups showed p = 0.479. The level of hemoglobin was 14.00±2.81 mg/dL in case group and 14.10±4.95 mg/dL in control group. The comparison of hemoglobin level between the two groups showed p = 0.905. The glucose level in this study was 137.43±40.51 mg/dl in case group whereas in control group was 123.58±20.31 mg/dl. The comparison of blood glucose between the groups showed p = 0.694 (Table 1). Most participants had hypertension with 82.69%. The comparison of hypertension in the two groups showed p = 0.233.

Table 1. Mean of clinical data

<table>
<thead>
<tr>
<th>Variable</th>
<th>National Institute of Health Stroke Scale</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case (n = 26)</td>
<td>Control (n = 26)</td>
</tr>
<tr>
<td>Age</td>
<td>55.43±9.05</td>
<td>53.12±11.96</td>
</tr>
<tr>
<td>Albumin</td>
<td>4.00±0.36</td>
<td>4.18±0.42</td>
</tr>
<tr>
<td>Sodium</td>
<td>134.12±3.38</td>
<td>135.12±2.52</td>
</tr>
<tr>
<td>Potassium</td>
<td>3.76±0.53</td>
<td>4.05±0.46</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>14.00±2.81</td>
<td>14.10±4.95</td>
</tr>
<tr>
<td>Blood glucose level</td>
<td>137.43±40.51</td>
<td>123.58±20.31</td>
</tr>
</tbody>
</table>

Hydration Status and the Stroke Severity

According to all participants in this study, 19.23% in case group experienced dehydration. However, control group was 9.62%. We did not find any significant difference, both statistically and clinically, between clinical severity degree of acute thrombotic stroke patients and dehydration condition and those with no dehydration. The p value was 0.126 and the odd ratio value was 2.625 (IK 95%; 0.748 – 9, 210; Table 2).
Table 2. Frequency of clinical data

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>National Institute of Health Stroke Scale</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Case (n = 26)</td>
<td>Control (n = 26)</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>17.31</td>
<td>32.69</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>32.69</td>
<td>17.31</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>Hypertension</td>
<td>44.23</td>
<td>38.46</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>5.77</td>
<td>11.54</td>
</tr>
<tr>
<td>Dehydration</td>
<td>Dehydration</td>
<td>19.23</td>
<td>9.62</td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>30.77</td>
<td>40.38</td>
</tr>
</tbody>
</table>

**Discussion**

The results which do not correspond with the proposed research hypothesis might be due to several things. First, the study did not include subjects suffering from ischemic stroke due to thrombotic in large vessels and therefore we did not get sufficient number of subjects into moderate to severe NIHSS groups with dehydration as well. In the studies conducted by Lin et al. and Schrock et al., the inclusion criteria included ischemic stroke patients due to large vessel disease 6,16. Second, this study did not limit the age of the subjects, causing that the proportion of subjects in the dehydrated group and the severity of clinical/NIHSS were not as expected. It is known that the elderly subjects are susceptible to dehydration which is a major cause of fluid and electrolyte disturbances 12. Third, this study did not consider other factors that might determine stroke prognosis, such as the location and width of the infarct. Stroke prognosis is affected by various factors including age, stroke severity degree, stroke mechanism, infarct location, comorbidity conditions, clinical finding, and related complications 20.

Cerebrovascular disease is one of the causes of morbidity, disability, and mortality worldwide. Generally the different genders are associated with stroke events. The relationship between age and stroke is more influential to men except at an advanced age. Increased age affects the morbidity of stroke, mortality, and the old outcome in both minor and major stroke. The age of over 65 years has a higher mortality risk in 2 months after stroke 21.

Hypoalbuminemia is a poor predictor of prognosis in patients with ischemic stroke, but the mechanism is still unknown. The currently known mechanism is not only limited to energy depletion, but also related to the damage of one’s immune and hormonal responses, as well as extracellular fluid expansion. In another study, the effect of infusion of albumin on ischemic brain of experimental animals was suggested. This is presumably because albumin has a neuroprotective effect mediated by various actions including antioxidative ability, affecting endothelial function, and venular perfusion 22.

Hyponatremia is a risk factor for stroke and cardiovascular disease. Mild hyponatremia is associated with increased mortality within 30 days of myocardial infarction and post-stroke mortality in 3 years. Hyponatremia is associated with acute mortality and worse outcome trends resulting in higher mortality within 12 months after stroke 23. The mechanisms of potassium effect on stroke are still unclear. Patients with lower potassium serum levels during stroke and after dismissed from hospital have a poor prognosis, especially in elderly patients 24.

Hypertension is a major risk factor for stroke. The increase of systemic blood pressure associated with the increased intra-cranial pressure (ICP), mainly due to suppression of the brain stem. It is strongly associated with intracerebral and subarachnoid hemorrhage, but this increase in blood pressure does not appear to be associated with cerebral ischemia 25.

The dehydration condition characterized by an increase in BUN/SCr ratio is a factor that can be used
to estimate poor outcomes in patients with chronic heart failure. The increase of BUN/SCr ratio is also reported to be associated with a neurological deterioration in ischemic stroke patients. A dehydration condition that increases the BUN/SCr ratio by more than 15 is at 2.2 times risk of clinical impairment in 30 days of post-onset of ischemic stroke. Infarction stroke patients with elevated BUN/SCr ratio had a higher prevalence for cardiembolic stroke compared to those without increased BUN/SCr ratio.

**Conclusion**

There was no correlation between hydration status assessed using BUN/Serum Creatinin ratio and the clinical severity degree assessed using NIHSS in acute thrombotic stroke. However, a further confirmation is still required.

**Ethical Clearance:** The study protocol was approved by the Ethical Commission to conduct basic science/clinical research in Dr. Soetomo General Hospital Surabaya, Indonesia. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, non-maleficence, and justice.

**Conflict of Interest:** The author reports no conflict of interest of this work.

**Source of Funding:** This study is done with individual funding.

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Prevalence of Benign Vocal Cord Disorder at Dr. Soetomo General Hospital Surabaya Indonesia in June 2015-June 2016

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1Department of Otolaryngology Head and Neck Surgery, Faculty of Medicine, Airlangga University, Dr. Soetomo General Hospital Surabaya, 60285 Indonesia

Abstract

Benign vocal cord disorder is a common case but its prevalence is difficult to determine. Reporting on the number of benign vocal cord disorder events is important for data management to arrange the effective management. Objective this research to determine the prevalence of benign vocal cord disorders, i.e. nodules, cysts, and vocal cord polyps. Method is a retrospective descriptive study conducted using medical record data of outpatient unit of Otolaryngology-HN division of larynx-pharynx Dr. Soetomo General Hospital Surabaya in the period of June 2015 to July 2016. Result benign vocal cord disorders consisted of 65.00% of subjects with nodules, 15.00% of subjects with polyps, and 20.00% of subjects with cysts. The ratio of male and female was 1:1. The most age was 20-59 years old with 60.00%. The largest occupational group is professional voice user with 60.00%. The location of benign vocal cord disorders was in 1/3 of the bilateral anterior as much as 85.00%. The most frequently used therapy is non-operative with 65.00%. The conclusion benign vocal cords disorder with the types of vodal, polyps, and vocal cord cyst occurred in 4.21% of all patients with dysphonic complaints. Vocal cord nodules had the highest incidence among other types of cord vocal diseases with 2.73% while the highest prevalence was shown by vocal cord nodules with 4.42%.

Keyword: Benign vocal cord, nodule, cyst, polyp

Introduction

Benign vocal cord are abnormal masses of excessive and uncoordinated laryngeal tissue 1. Benign vocal cord disorders decrease the efficiency of sound production. The voice becomes husky and breathy, causing patients to make more effort to talk. This leads to the patient complaining of tiredness and discomfort in the neck and throat. Patients with benign vocal cord disorder do not feel pain. The uneven surface of the vocal cords causes the mucus to get caught in the throat and cause them to react by coughing or clearing throat 2.

Benign vocal cord disorder can be divided into two: neoplastic and non-neoplastic. Non-neoplastic benign vocal cord disorders include vocal cord nodules, vocal cord polyps and vocal cord cyst 3. The talkative extrovert people and those whose works require the high use of voice are at risk of benign vocal cord disorders. Other risk factors include smoking, acid reflux, allergies and infections 4. Benign vocal cord disorder is a common case but its prevalence is difficult to determine.

In the United States from January 2004 to December 2008, the total population was fifty-five million patients. Of the total population, the prevalence of patients diagnosed with a dysphonia is 1%, and approximately 11% of dysphonia cases in the patients are caused by a benign vocal cord disorder. Benign vocal cord disorders consist of vocal cord polyps, vocal cord nodules, vocal cord abscess, vocal cord cellulitis vocal cord granuloma, and vocal cord leukoplakia 4, 5. There was never a certain rate of prevalence of benign vocal cord disorder in Dr. Soetomo general hospital. This study aims to
determine the prevalence of benign vocal cord disorder in Dr. Soetomo General Hospital Surabaya in the period of June 2015-June 2016.

**Method**

The subjects of the study were dysphonic patients treated at Dr. Soetomo General Hospital Surabaya, Indonesia that met the criteria of inclusion and exclusion. The inclusion criteria included patients with symptoms of dysphonia, patients diagnosed with benign vocal cord disorders, i.e. nodules, cysts, or vocal cord polyps, and patients with complete data. The exclusion criteria excluded patients with a history of malignancy.

This is a retrospective descriptive study conducted in outpatient unit of Otolaryngology-HN, Division of Larynx-Pharynx, Dr. Soetomo General Hospital Surabaya. The study materials were medical record data of outpatient unit of Otolaryngology-HN, Division of Larynx-Pharynx, Dr. Soetomo General Hospital Surabaya from June 2015 to June 2016. Prior to conducting the study, we have undergone ethical test at Dr. Soetomo General Hospital Surabaya, Indonesia. The initial number of samples before the identification based on the criteria was 475 subjects. After passing through the process, the number of subjects that fit the criteria was 20 subjects.

The data was collected by retrieving the data of new patients who came for a rigid and flexible laryngoscope action procedure from June 1, 2015 to June 30, 2016. The patients’ data were taken from the register book of larynopharyngeal division of 2015-2016 and the rigid and flexible laryngoscope report books of 2015-2016.

Patients’ data included patients’ identity, anamnesis, examination results, and diagnosis after rigid or flexible direct laryngoscope procedures. The collected data were arranged in tables according to age, sex, occupation, type of benign vocal cord disorder, location of benign vocal cord disorder, and the therapy given. The occupation types of the patients in this study were divided into 4 levels. Level I refers to elite vocal performer, i.e. singers and the radio announcers. Level II is professional voice user, i.e. lecturers. Level III is non-vocal professional, i.e. teachers, merchants, and students. Level IV is non-vocal non-professional, i.e. housewives and farmers. Data measurement results in this study are presented in the form of tables and descriptions.

**Result**

The number of female and male patients in this study was comparable with 50.00% for each group. Most patients were in the age group of 20-59 years old with 60.00%, followed by the age group of 1-9 years old with 20.00%. Most of the subjects work as teachers, traders, or students (level III occupations) with 60.00%, followed by housewives or farmers (level IV occupation) with 20.00%. Most subjects had benign vocal cord disorder in the form of nodule with 65.00% (Table 1).

Benign vocal cord disorders were grouped according to the location of the lesions, i.e. anterior 1/3 of the vocal cord, medial 1/3 of vocal cord, unilateral, and bilateral. The location of the vocal cord polyps was mostly in the anterior 1/3 of vocal cord, unilateral, with 10.00%, whereas the least was found in medial 1/3 of vocal cord, unilateral, with 5.00%. In the vocal cord cysts, most vocal cords were located at anterior 1/3 of vocal cords, unilateral, with 15.00%, and the least was found in medial 1/3 of vocal cord, unilateral, with 5.00%. The location of the vocal cord nodules was mostly located at anterior 1/3 of vocal cord, bilateral, with 55.00% and the least was found at anterior 1/3 of vocal cord, unilateral, with 5.00% (Table 2). Patients with benign vocal cords were grouped according to the type of therapy given. The most prevalent treatment group was the patients treated with non-operative at 65.00% and the least one was the group of patients who did not come for further therapy with 10.00% (Table 3).

<table>
<thead>
<tr>
<th>Variable</th>
<th>category</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td>Sex</td>
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<tr>
<td></td>
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</tr>
<tr>
<td>age (years)</td>
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<td></td>
<td>10-19</td>
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<tr>
<td></td>
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<td></td>
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<tr>
<td></td>
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<td>60.00</td>
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<tr>
<td></td>
<td>iv</td>
<td>10.00</td>
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<tr>
<td>Type of benign vocal cord disorder</td>
<td>nodules</td>
<td>65.00</td>
</tr>
<tr>
<td></td>
<td>Polyp</td>
<td>15.00</td>
</tr>
<tr>
<td></td>
<td>Cyst</td>
<td>20.00</td>
</tr>
</tbody>
</table>
Table 2. Distribution of the location of benign vocal cord disorders

<table>
<thead>
<tr>
<th>Vocal Cord Location</th>
<th>Anterior 1/3</th>
<th>Medial 1/3</th>
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<tbody>
<tr>
<td></td>
<td>Unilateral</td>
<td>Bilateral</td>
</tr>
<tr>
<td>Polyp</td>
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<td>-</td>
</tr>
<tr>
<td>Cyst</td>
<td>15.00</td>
<td>-</td>
</tr>
<tr>
<td>Nodules</td>
<td>5.00</td>
<td>55.00</td>
</tr>
</tbody>
</table>

Table 3. Therapy group and therapy results of patients with benign vocal cord disorders

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Non-operative</th>
<th>Operative</th>
<th>No Control Visit</th>
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</thead>
<tbody>
<tr>
<td>Polyp</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improved</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>remained</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>uncontrolled</td>
<td>5.00</td>
<td>-</td>
<td>10.00</td>
</tr>
<tr>
<td>Cyst</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improved</td>
<td>-</td>
<td>15.00</td>
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<td>remained</td>
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<td>5.00</td>
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<tr>
<td>uncontrolled</td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>Nodules</td>
<td></td>
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<tr>
<td>Improved</td>
<td>20.00</td>
<td>-</td>
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</tr>
<tr>
<td>remained</td>
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<td>5.00</td>
<td>-</td>
</tr>
<tr>
<td>uncontrolled</td>
<td>40.00</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Discussion

Benign vocal cord disorders are less common than the malignant ones. Benign vocal cord disorder can be divided into two: neoplastic and non-neoplastic. Non-neoplastic benign vocal cord disorders are due to infection, trauma, and degeneration. Some examples of non-neoplastic benign vocal cord disorders are vocal cord nodules, vocal cord polyps, and vocal cord cysts. Vocal cord nodules are always bilateral and almost symmetrical. Stroboscope examination will show a decrease in mucosal movement (mucosal wave). Voice therapy will decrease or relieve vocal cord nodules. Vocal cord polyps may occur unilaterally or bilaterally with exophytic lesions that are transparent or reddish in hemorrhagic polyps. Voice therapy does not change the size of the vocal cords polyps. Vocal cords cyst may be unilateral or bilateral. The location of vocal cord cysts is near the ligaments or within the subepithelial cavity. The subepithelial cavity is an area just below the vocal cord epithelium.

In this study we found the ratio of male and female patients with same benign vocal cord disorders. The same comparison of men and women in this study suggests that the risk of vocal cord disorders is the same in both women and men. Bastian et al. explained that benign vocal cord disorders are common in women because women use excessive sound more often. In the pre-menstrual period women experience premenstrual vocal syndrome (PMVS), described as the change of vocal cord stability due to hormonal fluctuation. PMVS is characterized by the inability to achieve high notes and the loss of power to make a voice. In this pre-menstrual period there is also a dryness of the larynx due to unbalanced estrogen and progesterone levels. A dry larynx encourages the patient to cleared his throat and this may lead to the occurrence of vocal cord nodules.
The age group of patients with benign vocal cord disorders in this study was mostly 20-59 years. Another study also mentioned the same thing. This explains why benign vocal cord disorders occur in patients in the working age group. The working age group consisted of individuals who actively work. This explains that patients in working age who often use voice will have a greater risk of suffering from benign vocal cord disorders.

VHI (Voice Handicap Index) score is a questionnaire containing 30 questions about the quality of life of dysphonic patients with good validity and reliability. VHI provides information about the patient’s perception on the level of sound disability in everyday life. A retrospective study by Behrman et al. proves that the level of voice needs related to lifestyle and occupation affects VHI scores. In patients with voice disorders, the higher level of the voice need leads to a higher VHI score. The high number of patients with working age in this study explains that patients in working age are in a high need of using the voice well. This also affects the VHI score. A high VHI score encourages the patients in working age to come for treatments.

The type of work has is closely related to the occurrence of benign vocal cord disorder. Those with the types of work requiring loud sounds with high frequencies are at risk factor for benign vocal cord disorders. The types of occupations that fall into level three tend to have the misuse of sound (vocal abuse) because in working, they use their voices as their primary resources without being professionally trained. The types of work included in level three require moderate sound quality but high voice load. The voice load include working hours, noisy environment, and inadequate work facilities. The excessive number of pupils in each classroom also affects the voice load of those working as teachers.

The most common type of benign vocal cord disorders in this case is the vocal cord nodules. The similar result was obtained in another study as well. Most patients with vocal cord nodules have a job in which voice becomes the primary requirement. This is why patients with a vocal cord nodules tend to come quicker for treatment compared those with other benign vocal cord disorders. Anterior 1/3 of the vocal cord is a membranous part. When the vocal cords vibrate, the membranous portion is the scene of friction and clash between the greatest vocal cords. Strong vibration that occurs for long periods of time causes vascular congestion accompanied by swelling in the membranous part of the vocal cord. This leads benign vocal cord disorders to be more commonly encountered at anterior 1/3 of vocal cords.

In this study, one patient experienced unilateral vocal cord nodules which was due to several possibilities. In this patient the vocal cord nodules were detected very early. Thus, the possibility of reactive lesions on the contralateral side of vocal cords has not been formed yet. The facilities for vocal cord examination used in this study were rigid and flexible laryngoscopes. Rigid and flexible direct laryngoscopes have limitations in detecting benign cord vocal disorders in detail. Unilateral lesions of the vocal cord may be detected by telelaryngoscope (stroboscope) as much as 79.8% whereas rigid direct laryngoscope with general anesthesia can only diagnose as much as 60.7%.

Non-operative therapy an effort to optimize the state of the larynx. The state of the larynx can be optimized using voice therapy by eliminating the habits that can injure the vocal cords, such as screaming or whispering, using voice with no exaggeration and optimal hydration. Other health problems associated with vocal cord irritation were also treated, such as acid reflux and control of allergies. If benign lesions of the vocal cord provide excellent response to non-operative therapy, then non-operative therapy is the best option to take.

Operative therapy is more intended for benign vocal cord disorders with the types of polyps and vocal cords cyst. Exophytic benign vocal cords disorders including vocal cord polyps and lesions that cause severe mucosal stiffness such as ligament cysts show poor results in non-operative therapy. Operative therapy becomes the primary choice if the patient has dysphagia associated with aspiration, has a risk of airway obstruction, and if there is suspicion of malignancy. For patients who have undergone non-operative therapy but do not get the expected results, then operative therapy can be taken into consideration. Operative therapy is also chosen if the need for voice for everyday life is very important for the patient. Non-operative treatment of the study included antibiotics, anti-inflammatory, steam inhalation, and voice rest. The operative therapy includes excision with rigid direct laryngoscope, excision with endoscope, and external excision. The percentage of patients treated non-operatively in the study was quite high and they...
showed good results 3.

**Conclusion**

This study was conducted at outpatient unit of Otolaryngology-NH, Division of Larynx and Pharynx, Dr. Soetomo General Hospital Surabaya from June 2015 to June 2016. There were 20 new patients with nodules, polyps, and vocal cord cysts for one year and the number was 4.21% of all patients with dysphonia complaints. Vocal cord nodules had the highest incidence among other types of cord vocal diseases with 2.73% while the highest prevalence was shown by vocal cord nodules with 4.42%.

**Conflict of Interest** : There is no conflict of interest

**Source of Funding** : This study is self-funded

**Ethical Clearance** : This study was approved by Ethical Comission of Health Research Faculty of Medicine University of Airlangga

**References**


Utilization of Deconcentration Health Funds in Planning, Budgeting, and Implementation in Indonesia

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1Center for Humanities Research, Development and Health Management, Ministry of Health, Indonesia

Abstract

Background: Deconcentration funds are funds that given to regions in achieving the target echelon 1 indicator of the main units in the ministry of health with program performance indicators, then translated into echelon 2 targets with the achievement of activity performance indicators. This deconcentration fund is one for health financing in achieving the National Medium-Term Development Plan (RPJMN) in the health sector.

Aim: to examine the allocation of deconcentrated funds in planning, implement, monitoring and evaluating.

Method: the secondary data document was analyzed for the allocation of deconcentrated funds, from the center in each division of the program. This research was conducted for 4 months from September to December 2018.

Result: the highest deconcentration funds from 2016 to 2017 were highest in the North Sulawesi provincial health department of 75.68% and 98.64%, while the lowest in the North Kalimantan provincial department was 39.25% and 68.03%. Whereas for 2018 the highest realization was in the Southeast Sulawesi provincial health department at 97.36% and the lowest in the Aceh provincial health department was 72.86%.

Conclusion: the deconcentration fund planning it is done top down and a menu of programs or activities has been determined. The amount of fund allocation has also been determined by the center, the regions only make and implement what has been made by the center. Activities in the area need to be monitored and evaluated both in substance and financial programs.

Keywords: deconcentrated funds, planning, monitoring and evaluation.

Introduction

Health is a basic human need to be able to live properly and productively, therefore it is necessary to conduct cost-controlled and quality-controlled health services1. Indonesia Sehat Program is implemented with 3 main pillars, namely the healthy paradigm, strengthening health services and national health insurance: 1) the pillar of the healthy paradigm is carried out with health mainstreaming strategies in development, strengthening preventive promotive and community empowerment; 2) strengthening health services is carried out with strategies to improve access to health services, optimize the referral system and improve the quality of health services, using a continuum of care approach and health risk-based interventions; 3) meanwhile the national health insurance is carried out with a strategy of expanding targets and benefits as well as quality control and cost control2,3.

The health budget allocation managed by the Ministry of Health in 2014 amounted to 50.35 trillion rupiahs with the realization of 47.58 trillion rupiahs. Large allocation and the realization of the budget has increased compared to 2014, namely the allocation of 38.64 trillion rupiah with the realization of 35.42 trillion rupiah. In terms of budget size, the increase also occurred
in the percentage of realization in 2014 compared to 2013, which was 91.66% in 2013 to 94.49% in 2014. The highest allocation of deconcentration funds in 2017 in West Java Province was Rp. 84,894,996,000 (6.2%) followed by East Java Province in the amount of Rp.83,610,717,000. (6.1%). While the fewest in the Province of DIY is Rp. 24,346,179,000. (1.8%).

In this study the emphasis is placed on an analysis of the use of deconcentration funds provided by the central government (Ministry of Health of the Republic of Indonesia) to Provincial Governments. Also seen in the process of planning, budgeting and implementation or use of deconcentration funds. The purpose of this study is to know the process of planning, budgeting, implementing and utilizing deconcentrated funds in health development. Besides knowing the allocation and realization and monitoring and evaluation of deconcentrated funds.

**Method**

In this study, a secondary data document was analyzed for the allocation of deconcentrated funds, from the center in each division of the program and information echelon 1 of the republic of indonesia’s ministry of health main unit, except for the health research and development agency and the inspectorate general, because the institution did not have deconcentration funds. besides conducting interviews with 78 planning officers of the Provincial Health Office in Indonesia. The implementation period is 4 months from December to December 2018. The ethical approval was taken from Health Research and Development Agency (Balitbangkes) of Ministry of Health Indonesia (No: LB.02.01/2/KE.231/2018).

**Results**

**Deconcentration Fund Allocation**

In general, the allocation has increased each year in each province from 2016 to Rp. 2,113,059,057,000, decreased in 2017 by Rp. 1,066,701,376.00, then in 2018 it increased to Rp. 1,102,942,700.000. -.

**Deconcentration Fund Planning**

The results of the study on the regional planning team from various provincial health department regarding the deconcentration fund planning process are as follows. According to respondents who were asked questions about the planning process stated that at most 61.0% was a combination of top down and bottom up, then followed by a top down of 28.6% and those with the least amount of bottom up was 10.4%.

**Synchronizing Planning**

From the results of interviews with the provincial department planning team, regarding synchronization of planning it was found that the majority were synchronous or matching with the Regional Budget (89.6%) and some were out of sync with the Regional Budget by 10.4%.
Figure 2: Synchronizing Planning with the Regional Budget

Budget Changes After Desk

In the deconcentration fund planning, after a desk with Echelon 1 Main Unit, according to the Provincial Health Service planning team as follows:

Figure 3: Budget Changes After Desk

Realization of the Use of Deconcentration Funds

The realization of deconcentration funds from 2016 to 2018 per Province in Indonesia can be seen from the following figure.
Deconcentration Fund Monitoring and Evaluation

Those who answered 26.8% were not monitored and evaluated, while 71.2% responded to it. From those who answered, monitoring and evaluation were carried out in one year as many as 67.7% with frequencies 1-2 times and 3.7% with frequencies 3-4 times.

Discussion

In planning the deconcentration fund from the center to the regions, it is always done using the top down method, meaning that the center has determined the menu of programs and activities, the region only follows and fills in the volume and amount that has been provided in the program menu. In the deconcentration fund planning there was no inclusion of APBD funds, but in the form of activities from other sections, for example the Health Promotion section by riding on health promotion socialization activities. The Provincial Health Office did not determine the program menu, but only had to carry out decisions from the center, although it could not be carried out at all. In the planning of deconcentrated funds, programs are not synchronized based on other funding sources, for example with the Regional Budget (APBD).
Deconcentration fund planning, correspondence and requests for documents needed to the center, then documents sent from the center. An internal meeting is held at the Farmalkes section, then by the center of each fund amount in each work unit, and this is permanent and cannot be changed\textsuperscript{10}. So that the menu does not match the needs of programs in the region, because there are too many programs from the central government but many cannot be implemented because the budget is insufficient, it seems to only pursue realization but does not guarantee program needs. The program holders are accustomed to referring to standard activities issued by the central government, so they forget the specific needs of the region. Even though the specific needs of the regions are different from those of the central government. This approach must be contextualized to the needs of the region even to the puskesmas through a system approach\textsuperscript{11}.

In the implementation of training activities sourced from deconcentrated funds, can not be on time, often late because the program implementers from the provincial health office must adjust to the schedule of resource persons from the Center, while in the Province there are other activities that are more priority. Determination of the management of deconcentrated funds by the Governor (Decree of Deconcentration Fund Managers) is often late, so the implementation of deconcentrated funds also experiences delays in achieving performance indicators both IKP and IKK. Even in the implementation of deconcentration funds, some are not in accordance with the needs of activities in the region, so what can not be financed by deconcentration funds will be allocated in planning by the provincial health office\textsuperscript{9,12}.

In the implementation of activities that use deconcentrated funds, for health human resource training activities, they have used standards created by the Center both the competency of the speaker / trainer, curriculum, participants, requirements, and certificates\textsuperscript{12}. The Provincial Health Office only implements programs or activities. In the implementation of deconcentration fund activities, absorption can be up to 70.0\%, because there are other activities carried out by the Governor’s Office that must be carried out first, but the Central Government stipulates that if the funds can be absorbed 85.0\% it can be said to be good\textsuperscript{13}.

Now facing the problem of accreditation of Puskesmas and Hospitals, all are required to be accredited, but deconcentration fund support is not in accordance with the accreditation target. BPJS will even break the cooperative relationship with hospitals that have not been accredited. Puskesmas accreditation is also being pursued, but with the highest accreditation status (Paripurna) it has not yet increased capitation from BPJS to puskesmas. This in-depth study was carried out, including the readiness of puskesmas and hospitals in achieving Universal Coverage (UC)\textsuperscript{14}. During this time there are training activities, in which the Center trains to the puskesmas which should not occur, meaning the Center conducts training at the Provincial level (master trainer), then the training is continued by Provincial master trainers at the Regency / City level, to the District and village. The central government conducts Norms, Standards, Guidelines and Decisions (NSPK) and conducts monitoring and evaluation after training both in substance and training mechanism\textsuperscript{15}. The implementation of the Kotamobagu City Government policy in the South Kotamobagu sub-district in free health services cannot be carried out optimally, this is due to the limited resources available, both medical personnel, and infrastructure facilities at the center Kotamobagu Selatan sub-district health services\textsuperscript{16,17}.

In monitoring and evaluating the use of deconcentration funds from the Central to the Province there are instruments used, and this is done once a year (annually), also seen from the realization of finance and output. There is a note, can also be seen the achievements for each health center that can be visited, the activities carried out with deconcentration funds\textsuperscript{18}. Also noted is the compatibility between program planning and implementation, for example the Farmalkes program menu in the Riau Islands, that from the Center monitoring and evaluation is conducted in 7 districts / cities (10 puskesmas and 10 pharmacies), but can only be done in 1 district with 3 puskesmas and 2 pharmacies, because the funds are insufficient. Report why and sent to the Center.

There is a monitoring and evaluation document for deconcentration funds at the central and provincial levels, there is a follow-up from monitoring and evaluation of the center to the regions, that is, the results will be processed whether the following year the activities listed in the menu are still feasible or not\textsuperscript{19}. Deconcentration funds can support the achievement of Minimum Service Standards (SPM), for example the use of high rational drugs, the use of antibiotics will go down, this can be budget efficiency (in accordance with programs and activities) but also to support the
activities of the Healthy Indonesia Program with the Family Approach (PIS-PK). For example: in the Riau Islands Province there is a cermat careful germa “which is” the movement of intelligent people using drugs”; this has been carried out in districts/cities, accompanied by cross-sector and cross-programs, the aim is to promote preventive and healthy community. In monitoring and evaluation it is necessary to prepare instruments and implementers in exploring problems until they find the root of the problem, then look for a solution to improve future programs. The monitoring and evaluation implementation is supported by quantitative data, which can provide strength and depth of analysis.20

**Conclusion**

The Study on the use of deconcentration funds in the planning, budgeting and implementation of the health sector is very important to determine the amount of ceiling, the planning menu and regional potential in carrying out activities that originate from deconcentration funds.

**Source of Funding:** This research was completely funded by Ministry of Health, Indonesia.

**Conflict of Interest:** There is no conflict of interest.

**References**


The Influence of Slow Stroke Back Massage, Cold-compress and Warm-compress to the Level of Prostaglandin F2α (PGF2α) in Primary Dysmenorrhea

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¹Lecturer, Nursing Science Program, Faculty of Health Science, Unipdu Jombang

Abstract

The cause of primary dysmenorrhea is excessive release of the hormone prostaglandin F2-alpha (PGF2α). The aim of this study was to assess the influence of SSBM, cold-compresses and warm-compresses toward the level of PGF2α in primary dysmenorrhea, using Post Test Only with Control Group Design and Pretest-Postest Control Group Design. The subjects were 76 female-student, selected by purposive sampling. NRS used to measure the pain level and ELISA used to measure PGF2α levels. The data were analyzed by using Paired Sample T-Test, One-Way Anova and Kruskal-Wallis test. SSBM, cold-compresses and warm-compresses are effective to decreasing of pain level and PGF2α levels in primary dysmenorrhea.

Keywords: Slow Stroke Back Massage, Cold-compresses, Warm-compresses, PGF2α, menstrual pain

Introduction

This incidence of dysmenorrhea is 20% to 90% among reproductive women and 15% occurred among young female followed with pain level around 2%-29%(2). Primary dysmenorrhea commonly occurred among adolescents without any pathological problems at the pelvis(3-4). The prevalence of primary dysmenorrhea among adolescent was 55.5-90.1%(1, 5). In Mexico, dysmenorrhea affects 65% of students’ daily activities(6).

The cause of primary dysmenorrhea is an increase in prostaglandin F2-alpha (PGF2α) production(7). The treatment of dysmenorrhea commonly used nonsteroidal anti-inflammatory drugs (NSAIDs). As we know medication consumption for long time will give some impact such as medication addictive(8-9), diarrhea, abdominal pain, nausea(10), kidney-complications, liver-complications, sleep-disorders(11), digestion-problems(12). The pharmacological treatment for dysmenorrhea was successful, but about 20-25% of the failure process(13). The one of the traditional treatment for dysmenorrhea is Slow Stroke Back Massage (SSBM), cold-compresses and warm-compresses. SSBM reduces pain and effectively increases endorphin levels(14). Cold-compresses reduce prostaglandins, so it will make strengthens the sensitivity of pain and other subcutaneous at the injury site by inhibiting the inflammatory process and giving analgesic(15). The skin stimulation causes endorphin loose so it will block blocking the transmission of pain stimulus. Skin stimulation activates the transmission of sensory A-Beta nerve fibers and decreases pain transmission C and delta-A fibers so the synaptic gate closes the transmission of pain impulse(16). Cold-compresses and warm-compresses are stimulates to loosing the endorphin β levels and regulate uterine hypercontractility during menstrual pain(17).

The results of previous studies indicated that Moxibustion consumption can reduce the levels of PGF2α, OT, vWF and increase the levels of β-EP (β Endorphin)(18). However, the influence of SSBM, Cold-compresses and Warm-compresses toward the intensity of pain and PGF2α levels in Primary Dysmenorrhea have not been clearly known. The aim of this study was to analysis the effect of SSBM, Cold-compresses and Warm-compresses toward Pain Intensity and Prostaglandin F2α (PGF2α) levels in Primary Dysmenorrhea.

Method

The design in this study was Post Test Only with Control Group Design and Pretest-Postest Control Group Design. The sample was 76 female students in faculty of health science of Unipdu Jombang who
experienced dysmenorrhoea, selected by purposive sampling. The samples were divided into six groups: SSBM (n=13), cold-compress (n=12), warm-compress (n=13), SSBM+cold-compresses (n=13), SSBM+warm-compresses (n=13), control (n=12). The data were collected by two instruments: NRS (Numeric Rating Scale) to measured the pain level, ELISA to measured PGF2α. Data were analyzed by Paired Sample T-Test, One-Way Anova and Kruskal-Wallis test.

**Findings**

Table 1. Intensity of menstrual pain before giving intervention

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSBM</td>
<td>6.31</td>
<td></td>
</tr>
<tr>
<td>Cold-compress</td>
<td>6.75</td>
<td></td>
</tr>
<tr>
<td>Warm-compress</td>
<td>6.31</td>
<td></td>
</tr>
<tr>
<td>SSBM+cold-compress</td>
<td>6.15</td>
<td></td>
</tr>
<tr>
<td>SSBM+warm-compress</td>
<td>6.77</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.17</td>
<td></td>
</tr>
</tbody>
</table>

*One-Way Anova, Post Hoc Tamhane’s test*

Table 2. The menstrual pain after giving intervention

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSBM</td>
<td>3.54</td>
<td></td>
</tr>
<tr>
<td>Cold-compress</td>
<td>2.83</td>
<td></td>
</tr>
<tr>
<td>Warm-compress</td>
<td>2.85</td>
<td></td>
</tr>
<tr>
<td>SSBM+cold-compress</td>
<td>2.23</td>
<td></td>
</tr>
<tr>
<td>SSBM+warm-compress</td>
<td>3.08</td>
<td></td>
</tr>
<tr>
<td>Positive-control</td>
<td>6.01</td>
<td></td>
</tr>
</tbody>
</table>

*One-Way Anova test, Post Hoc Tamhane’s test*

Table 3. The differences of menstruation pain between before and after intervention group

<table>
<thead>
<tr>
<th>Group</th>
<th>Before Mean±SD</th>
<th>After Mean±SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSBM</td>
<td>6.31±0.95</td>
<td>3.54±0.88</td>
<td>0.000</td>
</tr>
<tr>
<td>Cold-compress</td>
<td>6.75±1.29</td>
<td>2.83±1.19</td>
<td>0.000</td>
</tr>
<tr>
<td>Warm-compress</td>
<td>6.31±1.18</td>
<td>2.85±0.89</td>
<td>0.000</td>
</tr>
<tr>
<td>SSBM+cold-compress</td>
<td>6.15±1.28</td>
<td>2.23±0.09</td>
<td>0.000</td>
</tr>
<tr>
<td>SSBM+warm-compress</td>
<td>6.77±1.83</td>
<td>3.08±1.80</td>
<td>0.000</td>
</tr>
<tr>
<td>Control</td>
<td>6.17±1.40</td>
<td>6.00±1.21</td>
<td>0.166</td>
</tr>
</tbody>
</table>

*Paired Sample T-Test*

Table 4. The differences of PGF2α level between intervention group and control group

<table>
<thead>
<tr>
<th>Group</th>
<th>Median-pg/ml</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSBM</td>
<td>132.967</td>
<td></td>
</tr>
<tr>
<td>Cold-compress</td>
<td>136.033</td>
<td></td>
</tr>
<tr>
<td>Warm-compress</td>
<td>145.367</td>
<td></td>
</tr>
<tr>
<td>SSBM+cold-compress</td>
<td>151.300</td>
<td></td>
</tr>
<tr>
<td>SSBM+warm-compress</td>
<td>136.767</td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>156.544</td>
<td></td>
</tr>
</tbody>
</table>

*Kruskal-Wallis test, Post Hoc Mann-Whitney test.*

**Discussion**

The menstrual pain level before give the interventions were moderate to severe and after give treatments the average menstrual pain level in all treatment groups was mild and in control group was moderate level. The intensity of menstrual pain in this study was addressed with some previous studies which showed that the menstrual pain level among he respondents was moderate to severe level(19-20). Some risk factors of dysmenorrhea are include the early of age menarche, the longer of menstrual periods, the higher of menstrual volume, family history of dysmenorrhea, smokers, obesity, alcohol consumption(2,21-22), high
caffeine diet(23), and high levels of stress(24).

The results of this study showed that the average of menstrual pain level in all intervention groups are significantly decrease between pre-test and post-test. This showed that SSBM, cold-compresses and warm-compresses, SSBM+cold-compresses, SSBM+warm-compresses are effective to reduce the menstrual pain level. These interventions are cutaneous stimulation techniques, where the mechanism of these interventions in reducing pain level was explained according to the gate control theory. Gate control theory explained that skin stimulation can activate the transmission of the fibers of A-Beta nerve sensory(25). This process can reduce pain transmission through small diameter of C and delta-A fibers so the synaptic gate will closes the transmission of pain impulse. Generally, the cutaneous stimulation on the body is on the back and shoulders. Cutaneous stimulation are influenced peripheral fibers to send the impulses by using dorsal horn in the spinal cord, when the impulses carried by A-Beta fibers are dominate so the gate mechanism will close so the pain impulses are not delivered to the brain(26).

The average decreasion of menstrual pain level in SSBM-group. Touch and massage are the technique of sensory integration that affect the activity of nervous system. Individuals who perceived touch as a stimulation to relax, a relaxation response will appear. The use of appropriate cutaneous stimulation can reduce pain perception and muscle tension. Conversely muscle tension can increase pain perception(16). Massage of connective tissue makes relaxes on body, reduces muscle spasms, connective tissue tenderness, increases the circulation and β-endorphin plasma(27). This intervention can stimulate the autonomic nervous system to balance the sympathetic and parasympathetic nerves(28). The SSBM was effectived to reducing menstrual pain level(14). The connective tissue manipulation (CTM) can be used as an intervention to primary dysmenorrhoea and menstrual-related symptoms because this method didn’t give the potential effects such as analgesic, noninvasive and easy to do(29).

The average of menstrual pain level in cold-compress group was ranged 6.75-2.83. The intervention of cold-compress in the abdomen for 20 minutes by using a bag filled ice. The intervention of cold-compress to respondents was helpful to reduce the pain level, this because of the blood flow was decreased in area which is compressed, and gave analgesic effect by slowing the speed of nerve delivery so the pain impulses will be decrease or less to the brain. Cold-compresses was gave the physiological effects to reduce the inflammatory response, reduce blood flow, reduce edema, and reduce local pain(30). Cold therapy (ice compresses) intervention will be blocked and the impulses of pain will be reduce or loose for few times(26). Previous research results showed that cold-compresses was effective reduced menstrual pain(31).

After given of warm-compresses the average of menstrual pain significantly decreased. Warm-compresses is an intervention that can help to reduce pain by using dilated method of blood vessels so this will increasing the blood supply to the body(32). Some previous research said that warm-compresses was effected to reduce menstrual pain level(33-34).

In the control group after given management information about menstrual pain, a small proportion experienced a decreasing of menstrual pain level but was not significant. The information was included pharmacological and non-pharmacological menstrual pain treatment. Non-pharmacological treatment are regular exercise, adequate rest, warm-compresses on abdomen, yoga, warm water shower, massage, deep breath, acupunctur, acupressure, TENS, salt and sugar diet, cold-compresses and deuretics consumption (asparagus and watermelon). Pharmacological treatments are hormonal-drugs and NSAID(35).

The results of this study showed that there were no significant differences in PGF2α levels in all intervention groups, there were significant differences in PGF2α levels between intervention and control. Primary dysmenorrhoea caused by the increasing of prostaglandin production and the release of endometrial prostaglandins during menstruation so it will induce uterine hypercontractility, reduce uterine blood flow, and hypersensitive of pain(36). Menstrual cramps more worse because PGF2α was increased and PGE2 decreases, so primary dysmenorrhoea increased the ratio of PGF2α to PGE2(37-38).

Massage is a therapy to reduce the production of NF-κB, inflammatory cytokines and TNF-α(39). Inflammatory cytokines (ex: IL1-β, TNF-α) is stimulates prostaglandin production in the first day of menstrual phase(40-41). The results of previous studies indicated
that the Swedish Massage Therapy reduced the level of Mitogen-Stimulation of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-13, and IFN-γ(42). The decreasing of inflammatory cytokines impacted decreasing of prostaglandin stimulation production. Therefore, the SSBM intervention can reduce prostaglandin levels in primary dysmenorrhea.

Cold-compresses are the intervention which give vasoconstriction effects, prevented the edema, reduce inflammation, local anesthetics effect, reduce cell metabolism and increase blood viscosity. Warm-compresses are the interventions which give a vasodilation effects to blood circulation to becomes smooth and muscles relax(32). Hot stimulation can increase blood flow in blood vessels at uterus and consequently the prostaglandin vascular will be liquid, bradykinin, and histamine(25). Heat interventions in local area is as effective as NSAIDs(43). The NSAIDs mechanism to relieve primary dysmenorrhea depends on the inhibition of cyclooxygenase (COX), an enzyme which responsible for the prostaglandins production (and other prostanoids). The unbalanced amount of prostaglandins from the endometrium during menstruation are major cause of dysrhythmias contraction at the uterus, the reducing of local blood flow and the increasing of peripheral nerve sensitivity during Primary Dysmenorhea(44). Therefore, cold-compresses and warm-compresses are same effectively to reducing prostaglandins.

Conclusion

SSBM, cold-compresses and warm-compresses are effectively reduced pain level and PGF2α levels to dysmenorrhea.

Ethical Clearance-obtained from the ethics commission of Nursing Faculty of Airlangga University, Surabaya.

Conflict of Interest-no

Source of Funding-Directorate of Research and Community Service, Directorate General of Research and Development Strengthening, Ministry of Research, Technology and Higher Education.

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The Effect of Dexterity and Perturbation Exercise on Knee Osteoarthritis through Functional Balance and Power Improvement of Quadriceps and Hamstring

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Abstract

Backgrounds and Objective: Osteoarthritis (OA) is a set of joint disorders caused by different factors but has the same pathological changes. OA of the knee joint occurs mainly due to the tibiofemoral and patellofemoral joints as a continuous body weight support throughout human life both static and dynamic, thus gradually causing changes in the structure of the knee joint tissue. This study aimed to know the function and strengthening of muscle strength of quadriceps and hamstring in patient with knee osteoarthritis.

Method: Twenty-two patients with knee OA (average age of 51.7 years; range 40 to 65 years) were randomly grouped into intervention group (agility and perturbation exercises plus strengthening exercise of quadriceps and hamstring muscles) and the control group (strengthening exercise of quadriceps and hamstring muscles). Both group participated in a four-week exercise. Balance function, quadriceps and hamstring muscles strength (BBS, TUG, EN-Tree 1 RM) were assessed before and after intervention.

Result: The intervention and control group improved 1 RM quadriceps muscle (p = 0.0001) and 1 RM hamstring muscle (p = 0.0001) but there was no statistically significant difference between groups, score of BBS in intervention group improved significantly (p = 0.0001) and statistically increased significant between groups (p = 0.0001), score of TUG in intervention group improved significantly (p = 0.002) but there was no statistically significant difference between groups (p = 0.324).

Conclusion: Supplementation of agility and perturbation exercises on strengthening exercise of quadriceps and hamstring to balance function provided additional benefit with respect to the BBS score after four-week intervention of patients with knee osteoarthritis.

Keyword: knee osteoarthritis, perturbation, muscle strength, Berg Balance Scale, Time up and go test.

Background

Osteoarthritis is a joint disorder caused by different factors but has the same pathological changes. Osteoarthritis (OA) of the knee joint occurs mainly due to the tibiofemoral and patellofemoral joints as a continuous body weight support throughout human life both static and dynamic, thus gradually causing changes in the structure of the knee joint tissue. If the changes do not handle properly and appropriately it will develop into a chronic pathological process that causes damage to the entire structure of knee tissue.

Rehabilitation outpatient unit of Dr. Soetomo General Hospital Surabaya reported that the number of knee OA cases between October 2011 until April 2012 was 127 new cases and 3211 old cases. The high number of old cases was due to care phase and recurrence due to undiscipline in doing knee joint conservation program on damaged knee joints of their anatomical structure and function. Based on this report it was noted that knee OA...
is the second highest case after low back pain.

Some aspects of the human body such as vestibular system, vision, proprioceptive, muscle strength and cognition, have a relationship to the control of human balance thus it is an essential ability for everyday life. In knee OA there is a pathological change in the tissue structure within the joint space, ligaments, tendons, and periarticular tissues including muscles. This condition is followed by malfunction of the knee joint receptor mechanisms resulting a decreased proprioceptive function. On histological examination it was reported that the number of sensory receptors in the knee joint ligaments with OA decreased. Eldery with physiologically impaired proprioceptive function suffers from knee OA will further aggravate its proprioceptive function, thus it is estimated that this condition may cause an increase in the incidence of falling while walking or standing.

A study in 2002 found that the addition of dexterity and perturbation exercise in women aged 73 years with instability complaints on both knees due to OA during 12 sessions indicated that instability incidence did not continue and the patient could return to recreational activities such as walking, playing tennis and golf. The difference outcomes of both studies led researchers to examine the effects of adding dexterity and perturbation exercises on quadriceps and hamstring strengthening exercises to the improvement of proprioceptive function that controlled the balance thus it could improve the performance of the stability dynamics component of knee OA patients.

Method

This research was pre- and post-test control group design. This research was conducted in January 2013 until finished in Medical Rehabilitation Installation Unit of Dr. Soetomo Surabaya. The samples were new knee OA patients based on clinical symptoms and radiological examinations that met inclusion criteria and did not meet exclusion criteria. The sampling technique of this research was consecutive sampling method and then done a simple random sampling through lottery. Ethical appeals were submitted to the Ethics Commission for basic science/clinical research at Dr. Soetomo Surabaya.

The inclusion criteria were women aged 40 - 65 years; new knee OA sufferers who met the American College of Rheumatology clinical criteria of knee pain, less than 30 minutes of morning stiffness, crepitation when the knee was moved, tenderness on joint palate, swelling of the knee joint but not warm at palpation, new knee OA sufferers who met clinical criteria according to Kellgren-Lawrence radiology degree II and III, Berg Balance Scale (BBS) examination 41-56 = low risk fall, and 21-40 = moderate risk fall, examination of Time up and go test (TUG test) ≤20 = walking without aids, willing to participate in the research by signing the approval sheet after getting an explanation.

The exclusion criteria were patients with aids for ambulation, having fall history resulting in musculoskeletal disorders, patients with severe visual impairment, the patient had undergone total knee arthroplasty, patients with uncontrolled hypertension, patients with cardiovascular disease history, patients with nerve disorders resulting body weakness, and decreased sensibility and knee pain with VAS >3 with or without knee inflammation marks. Drop out criteria was arising knee pain during exercise with VAS >3.

The samples were randomly divided into two groups. Group 1 received strengthen training of quadriceps and hamstring muscles with EN-Tree tools plus dexterity and perturbation exercise twice/week every Tuesday and Thursday for 4 weeks, while group 2 received quadriceps and hamstring strengthening exercises with EN-Tree tools twice/week every Monday and Wednesday for 4 weeks. Pre- and post-exercise evaluation of balance function was done using BBS tool, TUG test and quadriceps muscle strength and hamstring 1 RM with EN-Tree. Data were analysed and compared their improvement of balance function and increased quadriceps and hamstring muscle strength before and after treatment.

The data were arranged in sheet and tabulated, then processed statistically using SPSS 21.0 (SPSS. Inc. Chicago IL). Normality test using one sample Kolmogorov-Smirnov test was done before comparing data between groups, homogeneity test was done using Levene test. Since the data were normally distributed and homogeneous, t 2 free samples were used to compare the data between groups, whereas paired t test was used to compare the data before and after the treatment.
Result

Table.1 Subject’s characteristic of respondent

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Treatment group (n=11)</th>
<th>Control group (n=11)</th>
<th>p*</th>
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<tbody>
<tr>
<td>Age (year)</td>
<td>51.7 ± 7.7</td>
<td>51.7 ± 4.8</td>
<td>1.0Φ</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>64.8 ± 13.4</td>
<td>67.2 ± 15.2</td>
<td>0.7Φ</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>157.3 ± 7.2</td>
<td>159.8 ± 5.9</td>
<td>0.4Φ</td>
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<tr>
<td>BMI (kg/cm²)</td>
<td>26.1 ± 4.7</td>
<td>26.2 ± 5.5</td>
<td>1.0Φ</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Sex</th>
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<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Male</td>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Grade</th>
<th>Treatment group (n=11)</th>
<th>Control group (n=11)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade OA</td>
<td></td>
<td></td>
<td>1.0€</td>
</tr>
<tr>
<td>Grade 2</td>
<td>7</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>4</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

Significant if p<0.05

Φ  t 2 free samples test
€ Chi-Square test

It could be seen from demographic characteristics in table above that treatment group that the youngest was 40 years and oldest was 65 years old with average of 51.73 ± 7.7 years, while in control group the youngest was 45 years and the oldest was 59 years with average of 51.73 ± 4.8 years. In the treatment group, the average weight was 64.82 ± 13.4 kilograms, while in control group was 67.18 ± 15.2 kilograms. In the treatment group, average height was 157.27 ± 7.2 cm while in control group was 159.82 ± 5.9 cm. In the treatment group, the average body mass index (BMI) was 26.1379 ± 4.7 while in control group was 26.22 ± 5.4. From the test result in table 1, it could be concluded that the research subject data according to age, body weight, body height, body mass index (IMT) was normally distributed.

Table.2 Comparison between control and treatment group on 1RM, BBS, and TUG to muscle

<table>
<thead>
<tr>
<th></th>
<th>Treatment group (n = 11)</th>
<th>p</th>
<th>Control group (n = 11)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td></td>
<td>Before</td>
</tr>
<tr>
<td>The association of average 1RM quadriceps</td>
<td>2.0 ± 0.3</td>
<td>9 ± 0.3</td>
<td>0.0001</td>
<td>2.2 ± 0.3</td>
</tr>
<tr>
<td>Comparison of 1 RM hamstring muscle</td>
<td>1.5 ± 0.5</td>
<td>2.68 ± 0.4</td>
<td>0.0001*</td>
<td>1.80 ± 0.4</td>
</tr>
<tr>
<td>Comparison of Average BBS</td>
<td>51.1 ± 2.3</td>
<td>54.3 ± 1.9</td>
<td>0.0001*</td>
<td>52.8 ± 1.8</td>
</tr>
<tr>
<td>Comparison of Average TUG</td>
<td>7.7 ± 0.9</td>
<td>7.1 ± 0.7</td>
<td>0.002*</td>
<td>7.6 ± 0.7</td>
</tr>
</tbody>
</table>
Table above shows homogeneity test with p <0.05 was done on age, body weight, height, BMI, gender and grade of knee OA to know diversity of demographic characteristics and to determine their effect on the research results. In both groups there were no significant differences in age, weight, height, BMI, gender and grade of knee OA.

The statistical test of the average increase of RM 1 quadriceps muscle before and after exercise in the treatment group showed a significant increase with p = 0.0001. In the control group also showed a significant increase with p = 0.000. Comparison of average RM 1 quadriceps muscle between treatment group and control group showed improvement, but did not show significant difference with p = 0.867. The statistical analysis of the average increase RM 1 hamstring muscles before and after exercise in the treatment group showed a significant increase with p = 0.0001. Comparison of average RM 1 hamstring muscle between treatment group and control group showed no increase and did not show significant difference with p = 0.901.

The BBS before and after intervention in the treatment group showed a significance with p = 0.0001. while in control group showed an increase but not significant with p = 0.341. The average BBS values between treatment group and control group showed a significant increase with p = 0.0001 The average of decreased TUG before and after intervention in the treatment group showed a significant decrease with p = 0.002. While in the control group showed a decrease but not significant with p = 0.324. The average TUG between treatment group and control group showed not significant decreased with p = 0.095

**Discussion**

The results shows that the increase in quadriceps and hamstring muscle strength in both groups was due to the training intervention using EN-tree pulley, and it had not shown the feedback mechanisms of proprioceptive organ sensitivity improvement. The results of this study were consistent with previous study in 2011, which examined 231 subjects with knee OA dividing into treatment groups receiving dexterity and perturbation exercise on strengthening exercises and control groups receiving only strengthening exercises. It was estimated that elderly with knee OA tended to avoid extreme movements that required balance and agility, thus the addition of perturbation and dexterity exercises did not give optimal results 12.

Based on subject’s occupation in treatment group, 5 subjects had sedentary lifestyles such as retirees, grandchildren sitter, housewives with maids, tailors, and workers with long sitting time in the office. Researchers argued that sedentary lifestyle classified in low-level activities that led to static body movements 13. This static condition made the mechanical function of knee joint neglected therefore the optimization of mechanoreseptor organ stimulation especially dynamic mechanoreceptors such as pacinian’s corpuscles through strain, pressure and burden of knee joint became minimum or lost 14.

It was argued that the short intervention period was relatively rapid for a rehabilitation process, therefore the impact of proprioceptive exercise and balance was not fully visible at the time of assessment. Another study supported the findings in this study that dexterity and perturbation exercise included in proprioceptive exercises had not shown an increase in quadriceps and hamstring muscle strength leading to increased functional activity 15.

A subsequent study of 63 knee OA patients performed joint proprioception assessment with joint motion detection threshold (JMDT), measurement of quadriceps and hamstring muscle strength with isokinetic dynamometer and functional assessment with 100 meters walking test and GUG 16. The effect of muscle weakness was stronger on the limitations of functional ability than in accurate proprioception. A study had not shown any feedback mechanism between proprioceptive function and muscle strength, although this had been proven by other study 17.

The BBS value improvement was in accordance with a study demonstrated the effect of perturbation exercise on parental balance function with a history of instability or fall. The subjects were divided into 2 groups consisting of those receiving the perturbation and control group who receiving flexibility and relaxation exercises. That study showed proprioceptive function improvement through perturbation exercises. It had also been proven in this study that the BBS score increased significantly indicating an improvement in proprioceptive function of subjects 18.
Conclusion

It was found that the addition of dexterity and perturbation exercise in muscle strengthening exercises twice a week for 4 weeks was more effective in improving the functional balance (BBS) than strengthening exercises alone of knee OA patients, the addition of dexterity and perturbation exercise in muscle strengthening exercises twice a week during 4 weeks was no more effective in improving the functional balance (TUG) compared to strengthening exercises alone in knee OA, the addition of dexterity and perturbation exercise in muscle strengthening exercises twice a week for 4 weeks was no more effective in improving quadriceps muscle strength and hamstring muscle compared to strengthening exercises alone in patients with knee OA.

Ethical Clearance: This research involves participants in the process using a questionnaire that was accordant with the ethical research principle based on the regulation of research ethic regulation. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, non-maleficence, and justice.

Conflict of Interest: The authors report that there is no conflict of interest related with this paper.

Source of Funding: This study is done with authors’ funding only.

References


Biocompatibility Test of Decellularized Cartilage Bovine Scaffold in Vitro and In Vivo

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¹Department of Orthopedics and Traumatology, Faculty of Medicine, Universitas Airlangga, Surabaya (60131), Indonesia

Abstract

Background: Incidence of cartilage defects was reported at 65.00% of the routine arthroscopy procedures. Dr. Soetomo Teaching Hospital Network Bank developed a cartilage scaffold from bovine that has been deselularized decellularized cartilage bovine scaffold (DCBS). It takes a study of biocompatibility test to prove that DCBS is not cytotoxic to cells and not generate a host response.

Objective: To analyze the biocompatibility of decellularized cartilage bovine scaffold (DCBS) in vivo and in vitro.

Methods and Materials: The subjects were experimental rats and experimental with in vitro and in vivo trials. The subjects were treated by toxicity test with MTT assay, irritation test using Draize Scale, acute/pyogenic systemic toxicity test by observing changes in body weight and temperature, and implantation test by observing fibrous capsule formation and Immunoglobulin G. Data was analyzed by using Kolmogorov-smirnov, Independent T-test, Mutivariate Analysis of Variance (MANOVA), and Mann Whitney.

Result: The percentage of viable DCBS (81.78%) and CBS (92.45%) was ≥70% of the control; thus, it is non-toxic to the cell. Draize scale in each group was in grade 0. Changes in body weight (p = 0.981) and temperature (p >0.05) had no significant association between groups. There was a significant difference mean of the fibrous capsule (p = 0.000) thickness and no significant difference in the mean number of Immunoglobulin G which was formed at week 1 (p = 0.87) and week 4 (p = 0.63) after implantation between CCBS and DCBS.

Conclusion: The biocompatibility test shows that DCBS is compatible as a biomaterial.

Keywords: DCBS, biocompatibility, In vivo, In vitro.

Introduction

Articular cartilage is a structure that coats the diarthrodial joint and serves to protect the subchondral bone from the axial force loading ¹. Damage to the articular cartilage, either due to trauma, degenerative, or due to congenital abnormalities causes joint pain and decreased quality of life ². Incidence of cartilage defects was reported at 65.00% of routine arthroscopy procedures. If lesions in the cartilage are not treated, it will cause joint pain, joint dysfunction and osteoarthritis (OA). Currently, there is no single effective therapy for osteoarthritis treatment ³.

Current treatment options are bone marrow stimulation (microfracture), osteochondral autograft transfer system (OATS) or mosaicplasty, and autologous chondrocyte transplantation (ACT) ¹. All of the above therapies do not produce enough hyaline cartilage tissue; thus, it continues on tissue engineering to develop strategies for cartilage repair and regeneration with biomechanical characteristics, biological compositions,
and the same organization as the original articular cartilage. Trias engineering network consists of 3 components, namely cell, signal, and scaffold.

Scaffold serves as a medium for closing defects and cell regeneration media. The cells used are mesenchymal stem cells. In vitro research proved that mesenchymal stem cell can regenerate and decellularized biomaterial scaffold limited the use of growth factor resulting in better cost and resource efficiency. Dr. Soetomo Teaching Hospital Network Bank developed a cartilage scaffold from bovine that has been deselularized (decellularized cartilage bovine scaffold (DCBS). Based on the deselulerization process, it is expected that cellular components that can induce an immune response can be eliminated and provided scaffold construction that may allow penetration of cultured cells. Therefore, we need to conduct a study of biocompatibility test to prove that the DCBS is not cytotoxic to the cell and not cause response from the host.

Methods

This study used an experimental study that was conducted in 3 months (November 2016-January 2017). Before being tested, replication was conducted by generating a large sample of 9 experimental rats in 1 group. In the in vitro test, samples of mesenchimal stem cells were divided into three groups, namely, a group of DCBS Sodium Dodecyl Sulfate (RSDS) products, a group cultured with Cartilage Bovine Scaffold (CBS), and a group cultured with media (control group). All three groups were tested by its viability using MTT Assay. In vivo trials, the experimental rats were divided into three groups: the implanted group of DCBS RSDS products, CBS implanted group, and placebo implanted group. Biocompatibility test results included irritation test, acute/pyrogenicity toxicity test, and implantation test.

The treatment stage was toxicity test with MTT assay (in vitro), irritation test (in vivo), acute/pyrogenicity toxicity test (in vivo), and implantation test (in vivo). In the cultured bone marrow mesenchimal stem cells (BM-MSCs), cytotoxicity test was added DCBS+MTT reagent on culture plate. Then, it was incubated within 48 hours at 37° C in an incubator containing 5% of CO2. After that, cell viability was quantitatively calculated based on the color change of the formazan. The tested material was not assessed by cytotoxic if the percentage of living cells was ≥70% compared to the control group.

The irritation test (in vivo) was performed by intracutaneous injection of a 0.2 ml scaffold extract on the back skin of experimental animals that had been shaved at 5 sites. Evaluation was conducted within 24 hours, 48 hours, 72 hours using Draize Scale on a scale of 0 (no erythema/edema) to 4 (severe erythema/edema).

Acute/pyrogenic toxicity test (in vivo) was performed by a 1x1x10 mm scaffold sample. This process subjects (experimental animals) were performed by intramuscular implantation. Thirty minutes before implantation was conducted rectal animal temperature measurement try. If there was a difference of 1° C from basal temperature or temperature over 39.8° C, the experimental animal was excluded from 61 toxicity tests. The evaluation was performed after implantation of 72 hours through weight and rectal temperature.

At the time of sample implantation (1x1x10 mm scaffold) inserted by injection with needle no. 16, the subjects were observed periodically at week 1 and week 4. The subjects were observed at the macroscopic implantation site (observation on fibrous capsule formation around the implant) and microscopic. Reactive materials can form capsules of 2-4 mm while non-reactive materials can form invisible capsules. For tissue sampling, the rats were tried to be terminated in every interval of examination. Then, tissue samples along with the implants were taken and inserted in a 10% formalin solution.

The data recorded were grouped, analyzed, and conducted normality test by using Kolmogorov-smirnov test, homogeneous data with Mutivariate Analysis of Variance (MANOVA) test, Independent T-test and Mann Whitney with SPSS program (SPSS, Inc., Chicago, IL).

Result

In acute systemic and pirogenisity toxicity test, subjects were performed weight measurements before and 72 hours after implantation. Average weight change was 9.67 gr, 9.33 gr, and 8.78 gr for DCBS, CBS, and control group respectively. There was no significant weight difference in all groups (p = 0.981) (Table 1).
The subjects were also performed rat temperature measurements before implantation, 30 minutes, 60 minutes, 90 minutes, 120 minutes, 150 minutes, and 180 minutes’ post implantation. The statistical test results showed no significant difference from the mean of temperature change in all groups (p > 0.05) (Table 2).

Table 3. One Way Anova Temperature Change Test (pyrogensity test)

<table>
<thead>
<tr>
<th>Group</th>
<th>One Way ANOVA (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 minutes</td>
</tr>
<tr>
<td>DCBS</td>
<td>0.502</td>
</tr>
<tr>
<td>CBS</td>
<td>0.511</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
</tbody>
</table>

In the implantation test, the average fiber fibrous caps were obtained at 1 week post implantation of 16.52 μ and 8.92 μ in Cellularized Cartilage Bovine Scaffold (CCBS) and DCBS. At week 4 post implantation, the average thickness of the fibrous capsule was 10.47 μ (CCBS) and 2.93 μ (DCBS). There was a significant difference mean of fibrous capsule thickness at 1 and 4 weeks post implantation between CCBS and DCBS (p = 0.000) (Table 3).

Table 3. Independent T-test of fibrous capsule thickness at week 1 and 4 post implantation

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean±SD body weight change</th>
<th>Independent T-Test (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 1</td>
<td>Week 4</td>
</tr>
<tr>
<td>CCBS</td>
<td>16.52±2.88</td>
<td>10.47±2.81</td>
</tr>
<tr>
<td>DCBS</td>
<td>8.92±2.38</td>
<td>2.93±0.84</td>
</tr>
</tbody>
</table>

Immunoglobulin G was performed on Immunoglobulin G test. The mean of Immunoglobulin G was formed at 1 week post implantation of 0.05 (CCBS) and 0.03 (DCBS). At week 4 after implantation, the average number of Immunoglobulin G in CCBS group was equal to DCBS of 0.04. There was no significant difference in mean Immunoglobulin G number at week 1 (p = 0.87) and week-4 (p = 0.63) after implantation between CCBS and DCBS (Table 4).
Table 4. Mann-Whitney U Test of the number of Immunoglobulin G at week 1 and week 4 post implantation

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean±SD Body weight change</th>
<th>Independent T Test (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 1</td>
<td>Week 4</td>
</tr>
<tr>
<td>CCBS</td>
<td>0.05±0.10</td>
<td>0.04±0.05</td>
</tr>
<tr>
<td>DCBS</td>
<td>0.03±0.05</td>
<td>0.04±0.05</td>
</tr>
</tbody>
</table>

**Discussion**

The cytotoxicity test shows a viable percentage of 70% of the control. The irritation test with Draize scale in each group is in grade 0. The acute toxicity/pyrogenicity test states that changes in body weight and temperature have no significant relationship between groups. The biocompatibility test that has been studied in previous studies on CBS showed that scaffolds have low toxicity levels in vitro and in vivo. It shows in vitro DCBS and CBS non-toxic to cells, according to the standard MTT assay that non-toxic in cytotoxicity test in vitro when the percentage of cells viable ≥70% of untreated controls.

The percentage of cell viability obtained in the DCBS group was fewer than the CBS group. This is probably because the SDS concentration used in this study is 5%, in contrast to previous studies where SDS used was 2%.

In another study, it was using 5% SDS, it also obtained a good microscopic deselularization result but because SDS is cytotoxic, it is necessary to conduct additional leaching which takes longer time with Phospat Buffered Salinese (PBS) for 4 days. However, this washing extension has an adverse effect on Extra Cellular Matrix (ECM) cartilage, which causes ECM to appear broken between the second and third days.

Irritation test in DCBS, CBS, and control group showed no irritation reaction in the form of edema and erythema in all three groups (draize score in all three groups were in grade 0). This shows that DCBS is a non-irritant material according to grading at ISO. Acute systemic toxicity and pyrogenicity test showed that DCBS, CBD. The control did not result in acute systemic toxicity and pyrogenicity reactions. This can be seen from the results of clinical observations of changes in body weight and rectal temperature of rats tested for 72 hours. There was no significant change in body weight and temperature between the 2 groups and the controls.

In this study, the evaluated implantation test was the fibrous and immunoglobulin tissue that was formed. In the evaluation of fibrous tissue of week 1 and week 4 showed that in the DCBS group, there was a better implantation test compared to CCBS which was characterized by smaller fibrous tissue formed in DCBS group although macroscopically both groups did not show any tissue reaction the formation of fibrous tissue in both groups both first week and second week does not exceed 2-4 mm. Previous studies comparing autologous ECM, allogenic ECM, xenogenic bovine collagen sponge (BCS), and synthetic scaffold (PLGA) implanted in rats showed that in the first week there was no fibrous tissue surrounding the autologous and allogenic ECM whereas in the BCS and PLGA groups formed the fibrous tissue that surrounds it.

The evaluation of the first week and the fourth week showed that the formation of Immunoglobulin G in CBS and DCBS groups. Despite the formation of Immunoglobulin G, statistically there is no significant difference. This suggests that CBS and DCBS do not generate a significant immune response but in the first week the number of Immunoglobulin G formed in the DCBS group was less than that of the CBS group.

The decellularization process is crucial in the elimination of cellular and antigenic components of tissue with the aim of preventing the transmission of the disease, reducing the immune response and inflammation, particularly in the xenogenic or allogenic donor tissue. Unlike cellular materials, ECM components are commonly found in all species. Therefore, this is tolerable enough when it is used as an allograft or xenograft. It can be seen that the DCBS used as xenograft did not result in a difference in the number of Immunoglobulin G depicting the absence of a meaningful immune response.

The previous studies of the biocompatibility of freeze-dried bovine cartilage in vivo in rabbits, it showed...
that the biomaterials were compatible and no significant immune response was obtained. Previous studies that examined the immunogenicity of chondrocytes and meniscus bovine and leporine also showed no immune response formed when chondrocytes and meniscus bovine and leporine were implanted in leporine. It is also seen that CBS and DCBS used as xenograft are also compatible and not stimulate the immune response. This study was comparing autologous ECM, allogenic ECM, xenogenic bovine collagen sponge (BCS), and synthetic scaffold (PLGA) implanted in mice, it showed that in the first week no macrophages were formed in all four groups.

However, the lowest was found in the autologous ECM group with the number of PLGA macrophages was equal to the number in the BCS group, but significantly larger than the allogenic ECM group. Besides that, this study also investigated the cytokines formed represented by interleukin-10 (IL-10), interleukin-2 (IL-2), interleukin-4 (IL-4), and TNF-α in the first week after implantation. From this study, it was found that IL-10 expression was lower in autologous ECM group than allogenic ECM, BCS, and PLGA. There was no significant difference in TNF-α transcription between the four groups although the number of TNF-α formed was lower in autologous ECM than in the other three groups. No IL-2 and IL-4 expressions were formed. It was concluded that autologous ECM stimulated the minimal cytokine against host response modulation.

**Conclusion**

The cytotoxicity test shows a viable percentage of 70% of the control. The irritation test with Draize scale in each group is in grade 0. The acute toxicity/pyrocillicity test states that changes in body weight and temperature have no significant relationship between groups. On the other hand, implantation test states that there is significant difference mean of fibrous capsule thickness and no significant difference mean of the amount of Immunoglobulin G which is formed at week 1 and week 4 post implantation between groups.

**Ethical Clearance**

This research involves animals in the process using a questionnaire that was accordant with the ethical research principle based on the regulation of research ethic regulation. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, non-maleficence, and justice.

**Conflict of Interest**: Authors guarantee that there is no conflict of interest of this paper in the future

**Source of Funding**: All of expenses for this research process are paid by authors without any sponsorship

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bubuk freeze dried bovine cartilage dengan platelet rich plasma (PRP) sebagai scaffold untuk regenerasi kerusakan tulang rawan secara in vivo pada hewan kelinci.


Predictors of Hospital Utilization Among Papuans in Indonesia

Agung Dwi Laksono¹, Ratna Dwi Wulandari²

Abstract

Hospitals were advanced referral health facilities or referrals for basic health services, it was very important for policymakers to know the factors that it was able to predict hospital use. The research objective was to determine the predictors of hospital use among Papuans in Indonesia. Data source from raw data of the 2013 Indonesia Basic Health Survey. Indonesia Basic Health Survey was a cross-sectional survey. The multi-stage cluster random sampling method was used to get 30,620 Papuan respondents. Multinomial Logistic Regression Test is used to determine the predictors of hospital utilization. There were 8 predictors of hospital utilization in outpatients used consisting of age, urban-rural area, gender, education level, socioeconomic status, insurance ownership, travel time and transportation costs, there were 5 predictor variables in hospital utilization in patients hospitalization consisting of gender, type of work, socio-economic status, travel time and transportation costs, and there were 2 predictors in hospital utilization in outpatient and inpatient care at the same time consisting of the cost of time travel and transportation to the hospital. Overall there were 9 predictor variables of hospital utilization in all categories (outpatient and inpatient).

Keywords: hospital utilization, healthcare, Indonesian basic health survey.

Background

Papua, which consists of two provinces, Papua and West Papua, is a province located at the easternmost tip of Indonesia. These two provinces fall into the backward category compared to other provinces located in the West. The provinces of Papua and West Papua often rank lower than many development performance indicators in Indonesia, including health development¹. Data-based health policy is needed to accelerate health development in these two provinces, including data on the utilization of health.

Hospital is a health service institution that organizes individual health services in a comprehensive manner that provides inpatient, outpatient, and emergency services. Hospitals are advanced referral health facilities or referrals for basic health services. The importance of hospitals as plenary services is the basis of the argument that it is very important for policymakers to know the factors that are able to predict hospital utilization². Evaluating the performance of the health care system, including hospitals, is important to support the performance of a better health system. It is the government’s obligation to ensure good access to hospitals for all communities without exception.

Based on the background, the purpose of this study was to determine predictors of hospital utilization among Papuans in Indonesia. It is hoped that the results of this study can become the basis for health policymakers to develop better policies in improving the access of Papuans to hospitals.

Materials and Method

The data used in this analysis was the raw data of the 2013 Indonesia Basic Health Survey. The Indonesia Basic Health Survey was a national scale survey with a multi-stage cluster random sampling method carried out by the Ministry of Health of Indonesia. The Indonesia Basic Health Survey was a five-year survey that was taken using a structured questionnaire.
The population was an Indonesian citizen who lives on Papua Island. The analysis unit in this study were respondents aged 15 years and over. It is assumed that at this age the respondent can make his own decision, to utilize the hospital or not. The sample size analyzed in this paper was 30,620 respondents.

The hospitals’ utilization was people’s access to hospitals, whether outpatient or inpatient. The time limit for outpatient was hospital use in the past month, while inpatient was the use of hospitals in the past year. It was assumed that the respondent can still remember the incidence of outpatient and inpatient properly.

Variable selection was done using the Chi-Square test to test the dichotomy variable, while the T-test was for continuous variables. This statistical test was used to assess whether there was a statistically significant relationship between the variables of hospital utilization as the dependent variable and the independent variable. There were 10 (ten) independent variables that will be tested as predictors of hospital utilization, namely age, the urban-rural area, gender, marital status, education, type of work, socioeconomic, insurance, travel time, and transportation cost to the hospital. The multinomial logistic regression test was used at the final stage to determine the variables that were predictors of hospital utilization among Papuans in Indonesia.

Findings

Bivariate analysis results show that there are significant differences between Papuans who utilize hospitals based on the category of urban-rural areas. On average Papuans who use hospitals are slightly older than those who do not use them. The results also show that Papuans who utilize hospitals are more women than men. This condition applies to all types of hospital utilization. Married people appear to dominate all types of hospital utilization. This difference is statistically significant.

Bivariate analysis results show that those who have educational status below the under primary school dominate hospital utilization. This result is in line with differences in utilization based on socio-economic status and type of work. The result illustrates that Papuans who utilize hospitals are dominated by very poor (quintile 1), and those who do not work. This condition is statistically significant. Three other variables, insurance ownership, travel time and transportation costs to the hospital, also had statistically significant differences in hospital utilization.

The results of the bivariate analysis showed that all variables tested were significant and could be continued with multinomial logistic regression. Table 1 shows the results of multinomial logistic regression tests to determine predictors of hospital utilization among Papuans in Indonesia. In this multinomial logistic regression test, “no utilization” was used as a reference.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Outpatient</th>
<th>Inpatient</th>
<th>Outpatient + Inpatient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>Lower Bound</td>
<td>Upper Bound</td>
</tr>
<tr>
<td>Age</td>
<td>1.008*</td>
<td>1.001</td>
<td>1.015</td>
</tr>
<tr>
<td>Area: Urban</td>
<td>0.667*</td>
<td>0.532</td>
<td>0.837</td>
</tr>
<tr>
<td>Gender: Male</td>
<td>0.782*</td>
<td>0.660</td>
<td>0.925</td>
</tr>
<tr>
<td>Marital Status: single</td>
<td>0.713</td>
<td>0.459</td>
<td>1.108</td>
</tr>
<tr>
<td>Marital Status: married</td>
<td>1.126</td>
<td>0.801</td>
<td>1.582</td>
</tr>
</tbody>
</table>
Table 1 shows that the predictor of hospital utilization in the outpatient category which proved significant there were 8 variables. The predictors are age, urban-rural area, gender, education level, socioeconomic status, insurance ownership, travel time and transportation cost. Table 1 shows that Papuan who lives in urban areas was 0.667 times using a hospital compared to Papuan who lived in rural areas (OR 0.667; 95% CI 0.532–0.837). It was also seen that Papuan males were 0.782 times compared to Papuan females in utilizing the hospital (OR 0.782; 95% CI 0.660-0.925). Papuan with an education level of under primary school 0.661 times using a hospital compared to college graduates (OR 0.661; 95% CI 0.470-0.930). Table 1 also shows significant socioeconomic status as a predictor of hospital utilization in the outpatient category. This significance applies to all socioeconomic statuses, at least the poorest Papuans used the hospital 2.637 times compared to the richest Papuans (OR 2.637; 95% CI 1.836-3.789). Papuan, who does not have insurance, uses 0.303 times compared to Papuan who has insurance that is managed by private. Papuan with travel time ≤30 minutes utilizing hospital outpatient 2.940 times compared to Papuan who had longer travel time (OR 2.940; 95% CI 2.349-3.680). Papuan with transportation costs of 15,000 IDR utilizing a hospital 1.465 times greater than Papuan which has more expensive transportation costs (OR 1.465; 95% CI 1.166-1.841). The exchange rate of 15,000 IDR equivalent to around 1 $ US.
The hospitals’ utilization in the inpatient category, there are 5 variables that proved significant as predictors. The predictors are gender, type of work, socioeconomic status, travel time and transportation cost. In the inpatient category, Papuan males were 0.711 times compared to Papuan females (OR 0.711; 95% CI 0.565-0.895). Papuan had a single status of 0.561 times compared to Papuan who was divorced (OR 0.561; 95% CI 0.314-1.004). Papuan who did not work 0.622 times used an inpatient compared to Papuan who had other categories of work (OR 0.622; 95% CI 0.401-0.965). The rich Papuans (Quintile 4) had utilized 1.561 times compared to the richest Papuans (Quintile 5)(OR 1.61; 95% CI 1.056-2.306). Papuans who have travel time to hospital ≤30 minutes utilize inpatient 1.458 times compared to those who have longer travel time (OR 1.458; 95% CI 1.503-2.018). Papuan with transportation costs of 15,000 IDR utilizing inpatient 1.560 times compared to Papuan which has more expensive transportation costs (OR 1.560; 95% CI 1.134-2.145).

Whereas in hospital utilization in the outpatient category and inpatient there are only 2 types of variables that are proven significantly as predictors. The two variables are time travel and transportation costs to the hospital. Papuans who have travel time ≤30 minutes 1.907 times use the hospital more than Papuan who has travel time >30 minutes (OR 1.875; 95% CI 1.056-3.329). While Papuan has a transportation cost of ≤15000 IDR 1.875 times more utilizing a hospital than Papuan who has transportation costs > 15000 IDR (OR 1.875; 95% CI 1.056-3.329).

The results showed that several demographic factors were found to be predictors of hospital utilization, namely age, gender, marital status, education level and type of work. Gender is a predictor in outpatient and inpatient categories. Papuans female utilizes hospitals more than Papuans male. Marital status partially predictors in the inpatient category. Education level partially predictor in outpatients, while types of work also found partially to be predictors in inpatient categories, but not in other categories. The study results that significantly showed demographic factors as predictors of hospital use were in line with research in Ghana4, German5, and Turkey6.

Socioeconomic status became a very convincing predictor in the outpatient category. This condition is in line with some of the results of other studies that examined the same thing7. Often socioeconomic is used as an indicator to evaluate the performance of the health service system to predict disparities8,9, inequity10, and inequality11.

Insurance ownership was also found to be a significant predictor in hospital utilization in the outpatient category. Those who do not have insurance have low utilization. The results of this study are in line with the objectives of the universal coverage policy launched by the Indonesian government to provide universal access to health services12. Health finance policies to improve public access are also carried out in several other countries by gradually implementing universal coverage. Evaluation of the implementation of financing policies in some of these countries shows positive results13, although there are still some obstacles to the implementation of financing policies in other conditions14.

Other predictors found were physical access variables indicated by travel time and transportation costs to hospitals. It is evident that better physical access makes better hospital utilization in outpatient and inpatient categories. These results are in line with the results of other studies in Indonesia15,16, and recent research in several other countries, including Iran17, China18, and Italia19. Pre-hospital factors are recommended in health economics studies because they significantly affect hospital accessibility, both in travel time and transportation costs20.

The results of this study cannot be separated from limitations, because this study is more superficial. Predict the phenomenon of surface hospital use. Need more in-depth research qualitatively to be able to better understand the reasons behind each phenomenon revealed in this study.

**Conclusion**

Based on the results of the study it can be concluded there were 9 predictor variables hospitals utilization (outpatient and inpatient) consisting of age, urban-rural area, gender, education level, work type, socioeconomic status, insurance ownership, travel time and transportation cost.

**Source of Funding:** Self-funding

**Conflict of Interests:** Nil

**Ethical Clearance:** During the data collection
process, informed consent was used with consideration of voluntary aspects, confidentiality, and procedures for collecting data. The 2013 Riskesdas has an ethical clearance certificate issued by the national ethics committee in NIHRD (code of ethics number: 01.1206.207).

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Hydration Status and Degree of Clinical Severity Patients with THRoMBotic Infarction Stroke

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Abstract

Background: Dehydration is common in acute ischemic stroke patients and associated with a poor increase in clinical outcomes. It is caused of the increase of hematocrit related to the infarct volume which is wider to the cerebra infarct patients. It is also related to the repeatedly emboli stroke and thromboembolic vena after the occurrence of the acute stroke.

Objectives: To analyze the correlation between hydration status and clinical severity in patients with acute thrombotic stroke.

Methods: This study was conducted by using control design consecutive admission sampling technique. Serum BUN and creatinine measurements were performed when patients were administered to the hospital. Dehydration was defined if the ratio of BUN/serum creatinine was >15. Patients were divided into dehydration and non-dehydrated groups. The degree of clinical severity was measured by using the National Institute of Health Stroke Scale (NIHSS) at 7 days of onset of stroke. Data were analyzed using chi square, fisher, t dependent test, and mann whitney (p <0.05).

Result: There were 19.23% stroke patients with dehydration were in the case group and the control group were 9.62%. There was no significant correlation between hydration status and clinical severity in acute thrombotic stroke patients (p = 0.126; OR = 2.625).

Conclusion: There was no correlation between hydration status and clinical severity in acute thrombotic stroke.

Keywords: Stroke, dehydration status, BUN/SK, NIHSS

Introduction

Stroke is the first leading cause of disability worldwide, the second most common cause of dementia, and the third leading cause of death. Stroke resulted in major clinical, social, and economic impacts with a total death rate of 6.2 million (±11% of the total). Around 9 million people suffered a stroke in 2008, 30 million people have had previous strokes and still alive. Stroke is the main cause of disability and this recent decline has fallen from the third leading cause of death to the fourth place in the United States ¹. The incidence of stroke has increased exponentially over the last 30 years and its causes vary by age. Ninety-five percent of strokes occur in people over the age of 45 and two-thirds of strokes occur in people over age 65. The risk of death in a person with stroke also increases with age. Stroke can occur at any age including the age of the children ².

Stroke is also a major cause of disability. It is approximately 55% of patients who survive up to 3 years will experience a decrease in quality of life. There is only 20% of patients with stroke can return to work as before ³,4. The association between renal function and stroke has been determined by a study assessing

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the association between hypertension and mortality with black patients suffering from acute stroke as the determinants. Stroke survivors have lower levels of urea than those who died. Friedman’s findings of stroke survivors in New Zealand mentioned that serum creatinin concentrations independently as predictors of mortality even after adjustment for confounding variables.

Euvolemia is important for adequate blood flow and maximizing oxygen intake into optimal tissue and organ function. Research showed blood flow to organs such as muscles and kidneys decreases significantly during dehydration. Cardiac output also decreases during dehydration. The brain has the ability to control blood flow through autoregulation that regulated constant cerebral perfusion pressure even when blood pressure changes. Dehydration has been shown to decrease the autoregulation response to orthostatic position changes, but in general does not decrease cerebral perfusion.

The role of dehydration in cerebral infarcts is thought to be multifactorial. Dehydration increases the viscosity of blood by decreasing blood flow to the brain through the decrease of intravascular volume. The increase hematocrit has been shown to be associated by a larger volume of infarction in patients with cerebral infarction. Dehydration has also been shown to be associated by recurrent embolic strokes and thrombotic events including venous thromboembolism following the onset of acute stroke. Dehydration can disrupt the distribution of oxygen to the brain, increase the risk of venous thromboembolism, and be associated with poor outcome in stroke. Dehydration can be detected by biomarkers of reduced fluid in the blood. Biomarkers that are often used to assess the presence of dehydration are the ratio of BUN/creatinine and plasma osmolality.

There is no hydration marker has been considered a golden standard, especially for mild dehydration. A total of 102 elderly patients were admitted to one of the educational hospitals who were diagnosed dehydrated. After a serum analysis, there is only 17% had an increase in serum osmolarity, 11% had elevated serum sodium, and 68% had an increase in blood urea nitrogen (BUN)/serum creatinine (SCr). BUN and SC are valuable laboratory parameters for evaluating renal function. The ratio of BUN to serum creatinine (BUN/SCr) is one of the useful laboratory indicators to determine the decrease in hydration status. Based on the description above, it is necessary to conduct an analysis of dehydration status and degree of clinical severity in patients with thrombolytic infarction. This study aimed to analyse hydration status of clinical severity and degree of clinical severity in patients with thrombolytic infarction.

**Method**

The participants in this study were all acute thrombotic stroke patients who met the inclusion and exclusion criteria. The inclusion criteria included patients diagnosed clinical acute thrombotic stroke and uncompromised CT scan, first stroke occurring within 24 hours, and having National Institute of Health Stroke Scale (NIHSS) score score of <4. NIHSS is a tool used to assess stroke damage with score range of 0 to 42. Exclusion criteria include patients having chronic renal failure, congestive heart failure, hemorrhagic stroke, embolism stroke, systemic inflammatory response syndrome, and use diuretic drugs. Participants who were willing to be involved in this study were required to fill the informed consent form in advance.

The participants were selected by identifying the number of patients with acute thrombolytic stroke in the previous year at Dr. Soetomo Teaching Hospital Surabaya, Indonesia. Afterwards, we found 101 participants with acute thrombolytic stroke who met the inclusion and exclusion criteria. During the course of the study, some patients with acute thrombolytic stroke were excluded from the study because the participant went home forcibly or died in ≤24 hours before blood sampling was taken for BUN and SCr examination. After those processes, we found 52 participants that were able to be enrolled in this study.

The procedure of this study involved patients diagnosed with acute thrombolytic stroke. Patients were examined by NIHSS on the seventh day. Participants were identified and divided into 2 groups, i.e. case group (moderate NIHSS with score 4-15) and control group (NIHSS with score <4). We conducted blood pressure measurement for the participants. Blood sample of 3 ml was stored in EDTA vials and identified for the BUN, SCr, Hb, Albumin, Potassium, Sodium, and Glucose. Assessment of dehydration status (BUN/SCr) was based on two studies. The criteria of hypertension refers to Joint National Committee 8.
The measurement data were analyzed according to the type of measurement results data using SPSS 22.0 (SPSS, Inc., Chicago, IL). Analysis on the participants’ gender data and blood pressure were conducted using chi square test (A p value of <0.05 was considered as statistically significant). Analysis on age, albumin, sodium, potassium, hemoglobin, and glucose data of the participants were conducted using t-dependent test (A p value of <0.05 was considered as statistically significant) with Kolmogorov Smirnov beforehand. The correlation between dehydration status and degree of clinical severity in stroke infarction patients was analyzed using chi square test (A p value of <0.05 was considered as statistically significant).

**Result**

**Sample Characteristic**

The demographic data in this study consisted of two types of data, i.e. gender and age. In case group, most participants were female (32.69%), while in control group most participants were male (32.69%). The comparison of age between the two groups showed p = 0.027 (Table 2). In case group, the mean of the participants’ age was 55.43±9.05 years old, while in control group was 53.12±11.96 years old. The comparison of age between the two groups showed p = 0.233 (Table 1).

The clinical data of albumin examination showed the value 4.00±0.36 mg/dl in case group and 4.18±0.42 mg/dl in control group. The comparison between the two groups showed p = 0.276. The sodium level was 134.12±3.38 mg/dL in case group and 135.12±2.52 mg/dL in control group. The comparison of sodium level between the two groups showed p = 0.653. The potassium level in case group was 3.76±0.53 mg/dL, while in control group was 4.05±0.46 mg/dL. The comparison of potassium level between the two groups showed p = 0.479. The level of hemoglobin was 14.00±2.81 mg/dL in case group and 14.10±4.95 mg/dL in control group. The comparison of hemoglobin level between the two groups showed p = 0.905. The glucose level in this study was 137.43±40.51 mg/dl in case group whereas in control group was 123.58±20.31 mg/dl. The comparison of blood glucose between the groups showed p = 0.694 (Table 1). Most participants had hypertension with 82.69%. The comparison of hypertension in the two groups showed p = 0.233.

<table>
<thead>
<tr>
<th>Variable</th>
<th>National Institute of Health Stroke Scale</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case (n = 26)</td>
<td>Control (n = 26)</td>
</tr>
<tr>
<td>Age</td>
<td>55.43±9.05</td>
<td>53.12±11.96</td>
</tr>
<tr>
<td>Albumin</td>
<td>4.00±0.36</td>
<td>4.18±0.42</td>
</tr>
<tr>
<td>Sodium</td>
<td>134.12±3.38</td>
<td>135.12±2.52</td>
</tr>
<tr>
<td>Potassium</td>
<td>3.76±0.53</td>
<td>4.05±0.46</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>14.00±2.81</td>
<td>14.10±4.95</td>
</tr>
<tr>
<td>Blood glucose level</td>
<td>137.43±40.51</td>
<td>123.58±20.31</td>
</tr>
</tbody>
</table>

**Hydration Status and the Stroke Severity**

According to all participants in this study, 19.23% in case group experienced dehydration. However, control group was 9.62%. We did not find any significant difference, both statistically and clinically, between clinical severity degree of acute thrombotic stroke patients and dehydration condition and those with no dehydration. The p value was 0.126 and the odd ratio value was 2.625 (IK 95%; 0.748 – 9, 210; Table 2).
### Table 2. Frequency of clinical data

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>National Institute of Health Stroke Scale</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Case (n = 26)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>17.31</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>32.69</td>
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<tr>
<td>Blood pressure</td>
<td>Hypertension</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>5.77</td>
<td></td>
</tr>
<tr>
<td>Dehydration</td>
<td>Dehydration</td>
<td>19.23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Normal</td>
<td>30.77</td>
<td></td>
</tr>
</tbody>
</table>

### Discussion

The results which do not correspond with the proposed research hypothesis might be due to several things. First, the study did not include subjects suffering from ischemic stroke due to thrombotic in large vessels and therefore we did not get sufficient number of subjects into moderate to severe NIHSS groups with dehydration as well. In the studies conducted by Lin et al. and Schrock et al., the inclusion criteria included ischemic stroke patients due to large vessel disease. Second, this study did not limit the age of the subjects, causing that the proportion of subjects in the dehydrated group and the severity of clinical/NIHSS were not as expected. It is known that the elderly subjects are susceptible to dehydration which is a major cause of fluid and electrolyte disturbances. Third, this study did not consider other factors that might determine stroke prognosis, such as the location and width of the infarct. Stroke prognosis is affected by various factors including age, stroke severity degree, stroke mechanism, infarct location, comorbidity conditions, clinical finding, and related complications.

Cerebrovascular disease is one of the causes of morbidity, disability, and mortality worldwide. Generally the different genders are associated with stroke events. The relationship between age and stroke is more influential to men except at an advanced age. Increased age affects the morbidity of stroke, mortality, and the old outcome in both minor and major stroke. The age of over 65 years has a higher mortality risk in 2 months after stroke.

Hypoalbuminemia is a poor predictor of prognosis in patients with ischemic stroke, but the mechanism is still unknown. The currently known mechanism is not only limited to energy depletion, but also related to the damage of one’s immune and hormonal responses, as well as extracellular fluid expansion. In another study, the effect of infusion of albumin on ischemic brain of experimental animals was suggested. This is presumably because albumin has a neuroprotective effect mediated by various actions including antioxidative ability, affecting endothelial function, and venular perfusion.

Hyponatremia is a risk factor for stroke and cardiovascular disease. Mild hyponatremia is associated with increased mortality within 30 days of myocardial infarction and post-stroke mortality in 3 years. Hyponatremia is associated with acute mortality and worse outcome trends resulting in higher mortality within 12 months after stroke. The mechanisms of potassium effect on stroke are still unclear. Patients with lower potassium serum levels during stroke and after dismissed from hospital have a poor prognosis, especially in elderly patients.

Hypertension is a major risk factor for stroke. The increase of systemic blood pressure associated with the increased intra-cranial pressure (ICP), mainly due to suppression of the brain stem. It is strongly associated with intracerebral and subarachnoid hemorrhage, but this increase in blood pressure does not appear to be associated with cerebral ischemia.

The dehydration condition characterized by an
increase in BUN/SCr ratio is a factor that can be used to estimate poor outcomes in patients with chronic heart failure. The increased of BUN/SCr ratio is also reported to be associated with a neurological deterioration in ischemic stroke patients. A dehydration condition that increases the BUN/SCr ratio by more than 15 is at 2.2 times risk of clinical impairment in 30 days of post-onset of ischemic stroke. Infarction stroke patients with elevated BUN/SCr ratio had a higher prevalence for cardiembolic stroke compared to those without increased BUN/SCr ratio.

**Conclusion**

There was no correlation between hydration status assessed using BUN/Serum Creatinin ratio and the clinical severity degree assessed using NIHSS in acute thrombotic stroke. However, a further confirmation is still required.

**Ethical Clearance:** The study protocol was approved by the Ethical Commission to conduct basic science/clinical research in Dr. Soetomo General Hospital Surabaya, Indonesia. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, non-maleficence, and justice.

**Conflict of Interest:** The author reports no conflict of interest of this work.

**Source of Funding:** This study is done with individual funding.

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The Effect of Allogenic Bone Marrow Mesenchymal Stem Cell - Platelet Rich Plasma (Bmecs - Prp) Intra-Articular Injection Effect on the Regeneration of Full-Thickness Joint Cartilage Defect on Rabbit

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Abstract

Introduction: Full thickness cartilage defects are still a problem until present since the handling has not give any satisfactory results. Current handling is performed through cartilage engineering using mesenchymal stem cells alone and or in combination with growth factor. The purpose of this study was to investigate the effect of injection of intra-articular injection of Allogenic bone marrow mesenchymal stem cell - Platelet rich plasma (BMSCs-PRP) on regeneration of full thickness cartilage defect in rabbits.

Method: The design of this study is a post-test only control group design using 36 New Zealand white rabbits divided into three groups treated with PRP, BMSCs and BMSCs-PRP. Results are evaluated after 10 weeks.

Results: In the evaluation, macroscopic images showed the best healing in the BMSCs-PRP group. Histopathologic examination showed that in the MSCs-PRP group there was a significant increase in the amount of chondrocytes (p = 0.000), cartilage area (p = 0.000), as well as the number of Agecoprogenitor expec- tion cells (p = 0.000) and type 2 collagen (p = 0.000).

Conclusions: Intra-articular injections Allogenic bone marrow mesenchymal stem cell (BMSCs-PRP) is able to regenerate and cure full-thickness joint cartilage defects through differentiation of MSCs into condroblasts.

Keywords: Allogenic, Bone marrow Mesenchymal stem cell, Cartilage, Platelet rich plasma, Full-thickness

Introduction

Cartilage of joints is an important part of the joint component. Damage to joints often occurs in sports activities, where some studies get nearly 49% of joint damage occurring due to sports injuries. Its high ability to hold and absorb weight is helpful in resisting the mechanical forces acting on joints during sports activities 1. In the destruction of joint cartilage, the healing of the formed tissue is very fragile because it consists of fibrocartilage so that even small defects will cause degeneration over time, eventually leading to osteoarthritis 2.

The affected cartilage of the affected joints shows limited healing ability. Damage to joint cartilage that affects the subcondral bone shows signs of healing due to the release of bone marrow mesenchymal stem cells (BMSCs). This is the principle of microfracture. Current treatments such as arthroscopic management, autologous
osteochondral transfer, autologous chondrocyte implantation (ACI) all showed better results although a systematic review comparing the three methods found no consistent therapy method showed better results than other techniques ³.

Im et al. (2001) examined the ability of bone marrow mesenchymal stem cells (BMSCs) to treat defects in cartilage. BMSCs suspension is given on Ham F-12 medium before being injected into full thickness cartilage defect in the rabbit patella basin area. Evaluation after 14 weeks showed the BMSCs group resulted in full cure in the subcondral bone tissue layer. Meanwhile in the control group the healing tissue is thinner, irregular and undifferentiated with a little bit of collagen type 2 matrix. The use of BMSCs with this method improves cartilage repair although the cure is not the same as the natural content ⁴.

The previous researcher examined the ability of bone marrow mesenchymal stem cells (BMSCs) to treat defects in cartilage. BMSCs suspension was given on Ham F-12 medium before being injected into full thickness cartilage defect in the rabbit patella basin area. Evaluation after 14 weeks showed the BMSCs group resulted in full cure in the subcondral bone tissue layer ⁵. In general, mesenchymal stem cells to be implanted in joint cartilage defects are included in the three-dimensional scaffold. Some issues relating to the use of scaffolds are material selection, the ability to support cell viability and differentiation, retention problems and research have found that PRP administration has a regenerative effect because it proves to improve the healing of osteochondral defects in rabbits ⁶.

The administration of PRP to MSCs cultures can increase in vitro proliferation and there is a tendency to increase MSCs differentiation into condroblasts and osteoblasts. Allogenic MSCs can increase cartilage regeneration and do not cause autoimmune rejection reactions. MSCs have immunosuppressive syphles that allow them to be used allogenically.

Method

This is a true-experimental study with post-test only control group design. This study protocol has been approved by the Ethics Committee of Faculty of Veterinary Medicine Universitas Airlangga Surabaya. The inclusion criteria of this study were male rabbits, adulthood 6-8 months, body weight 2.5 - 3.5 kg. Exclusion criteria were infection, knee injuries and rabbits died before 8 weeks. The initial stage in this research is to prepare the manufacture of mesenchymal stem cells-platelet rich plasma (BMSCs - PRP) as follows; bone marrow aspirations for making Bone marrow mesenchymal stem cells (BMSCs); culture and expansion of bone marrow-mesenchymal stem cells (BMSCs) from the bone marrow; characterization of Bone marrow-mesenchymal stem cells (BMSCs); making platelet rich plasma (PRP); making cartilage defects in the knee joint; intraarticular injection of cartilage defect in the knee joint; Sacrifize rabbits at 12 weeks; evaluation of histological and immunohistochemical examinations and evaluation of data ⁷.

All the collected data were tabulated and statistically processed using SPSS (SPSS. Inc. Chicago IL). This research were conducted through data descriptive analysis. Normality data test was conducted to know the normally distributed data in all data of the study result. Homogeneity test of variance was done for the normally distributed data ⁸. The quantitative data comparative test result of eosin hematoxylin (HE) examination of the amount of chondrocytes and cartilage area was performed using one way analysis of variance (ANOVA) test on the data with homogeneous variance to know the difference of the examination result data in the three treatment groups ⁹. The quantitative data comparative test result of immunohistochemical examination (IHC) number of agrecan expression condenser and collagen type 2 using one way analysis of variance (ANOVA) test on data with homogeneous variant to know difference of examination result data in all three treatment groups. In the abnormally distributed data, statistic test was performed with Kruskal Wallis test ¹⁰.

Results

To obtain sufficient amount of BMSCs for $2 \times 10^7$ injections, culture was carried out for 2 weeks. This amount was achieved at passage 3. The optimal number of BMSCs for mobilization to the defect at the rabbit knee was $1 \times 10^7$ per mL (Figures 1a and 1b). Platelet rich plasma (PRP) was made with two rounds of 3000 rpm for 13 min at 1st and 3000 rpm for 15 minutes at stage 2. The final result of 20 ml of peripheral blood taken and processed was 2 ml PRP. In PRP an increase in platelet count was 5x more than the platelet count in peripheral blood (Figure 1c and d).
In groups that received BMSCs-PRP the defect was almost invisible. The defect area has been filled with the same whitish color cartilage tissue as the surrounding cartilage color. The boundary of the healing area with its surroundings is not clear with the same surface height as the surroundings. This shows the best healing occurs in the BMSCs-PRP group (Figure.2). The area of cartilage in each treatment group. The results of analysis with Brown-Forsythe Statistic obtained p value = 0.000 indicating a difference of cartilage area in PRP, BMSCs and BMSCs-PRP groups. The width of cartilage in each treatment group. The result of analysis with Brown-Forsythe Statistic obtained p value = 0.000 indicating a difference of cartilage area in PRP, BMSCs and BMSCs-PRP group (Figure.3).

The difference in numbers was seen in the agrecan expression of different cells between tissue matrices in each treatment group using x100 magnification. Based on the number of Agreecan expression cells it is found more in the BMSCs-PRP group (Figure 3E) than in the PRP and BMSCs groups. As for the difference of Agreecan expressor cells with core and were not shown with positive or negative explanation at x400 magnification. BMSCs-PRP group (Figure 3F) had the most positive cells compared to PRP group (Figure 3B).

Immuno histochemical examination of collagen type 2 in the figure above shows the difference in the number of collagen type 2 expression cells in each group. The amount difference can be seen in the network matrix in each treatment group using x100 magnification. The number of collagen type 2 expression cells was found more in the BMSCs-PRP group (Figure 3E) than in the PRP group (Figure 3A) and BMSCs (Figure 3C). At x400 magnification, each treatment group expressed a condroprogenitor cell. (Figure 3B, D and F).
Discussion

The procedure of this study was carried out according to the previous research results where the process of centrifugation done 2 times with speed 3000 rpm for 13 minutes at 1st and 3000 rpm for 15 minutes in the second round. In another study another commercial system was used where leukocytes-PRP were classified according to Dohan Ehrenfest. In this system PRP was produced with platelet concentration of about 220,000 platelets/μl. Preparation of PRP consists of a stage where the poor platelet plasma (PPP) was not disposed.

The advantage of this process is to avoid excessive manipulation that can lead to platelet stress in the second centrifuge and avoid removing growth factors that depend on PPP. Another advantage is the closed circuit system in its purification process which causes the procedure to be safer. In this process usually after centrifugation of 8 ml of peripheral blood, platelet recovery> 95% and recovery of leukocyte> 58% (mononuclear cell recovery 93%) in 4 ml PRP 11.

The platelet count levels in this study may provide optimal benefits. The concentration of platelets in PRP in this study did not differ greatly with the Anitura study which obtained platelet concentration results of about 2.5 times normal. Other studies have shown that an excessively high platelet count can decrease the expected effect (paradoxsal effect). The dose-response relationship between platelet concentration and the stimulated biological process remains unclear. Once the growth factor reaches the targeted receptor surface, the additional growth factor concentration has no effect. Growth factor will decrease the effect if the required upper (high) concentration limit is reached12.

In an intra-articular injection study of BMSCs on osteoarthritis raised by bone debris and cartilage from osteochondral fragments, there was a significant decrease in postaglandin levels in the synovium fluid.13 This effect is not found when an injection of MSCs is sourced from fat. In this study there was an increase of tumor necrosis factor (TNF) levels in the synovium fluid. The effects of BMSCs on cartilage improvement are derived from inhibition of catabolic effects and stimulation of anabolic effects through cytokine mediators 14.

A recent study on horse experimental animals for 2 to 5 years found that BMSCs produce protein core and
chondroitin sulfate with longer chain and few shorter protein molecules than chondrocytes. This shows that BMSCs produce aggrecan with younger phenotype characteristics than chondrocytes produced. These results are in accordance with the results of this study where there is an increase in aggrecan content in defects given BMSCs 15.

Allogenic BMSCs are a new hope for healing cell-based cartilage. Allogenic BMSCs allow patients to be injected only once, no longer needing 2 actions for harvesting cells and implantation. This allogenic technique makes the treatment of defects in cartilage to be reliable, easy to apply and not invasive 16.

Conclusion

Intra-articular injection of allogeneic bone marrow mesenchymal stem cell - rich plasma platelet (BMSCs-PRP) was able to increase the differentiation of BMSCs into condroblasts. It creates formation of more aggressive and collagen type 2 expression cells than those injected only with BMSCs or PRP alone in fullthickness joint cartilage defects. PRP is able to form an environment suitable for MSCs so that MSCs proliferate and differentiate into more condroblasts.

Ethical Clearance

This research involves participants in the process using a questionnaire that was accordant with the ethical research principle based on the regulation of research ethic regulation. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, non-maleficence, and justice.

Conflict of Interest : The authors report no conflict of interest related to this paper so far

Source of Funding : All of expenses of this research are paid by authors only without external sources

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High LDL levels lessen bone destruction during antigen-induced arthritis by inhibiting osteoclast formation and function. BMJ Publishing Group Ltd; 2019.


Health Wisdom in the Traditional Healing of the Panaragan Java Subethnicity: The Case Study of Warok Ponorogo

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Abstract

The Panaragan Java Subethnicity has a customary leader called warok. This leader is known for his ability to possess supranatural powers which may heal diseases. This traditional skill is obtained from the ancestors. It uses natural materials complete with the mantras. The aim of this research is to know the local wisdom in the field of health, especially in the art of Reyog from Ponorogo. This research uses a descriptive-qualitative design. The data is obtained through the technique of observation and interview. The results show that there are three types of health wisdom in the Panaragan subethnicity which are the wisdoms of health profession, medicine, and healing.

Keywords: Health Wisdom, Warok, and Ponorogo

Introduction

The province of East Java, Indonesia, has ten cultural subareas which are Mataraman Java, Panaragan Java, Arek, Samin (Sedula Sikep), Tengger, Oising (Using), Pandalungan, Madura Pulau, Madura Kangean, and Madura Bawean.(1) These ten cultural subareas have various cultural characters which are different from one and another. These variations exist in the language, the arts, and the culture. One of the interesting cultural subareas is the Panaragan subarea.

According to a Western observer,(2) the Panaragan Java subethnicity is included in the Mataraman subarea which includes Madiun, Magetan, Trenggalek, Pacitan, and Ngawi. This is different from the opinion of an Indonesian researcher(3) who suggests that the Panaragan subarea is separate from the Mataraman subarea. The justification is that the Panaragan ethnicity subarea has a different language, traditional costumes, and traditional arts. It is also an autonomous area which was formerly known as the Wengker area before there was an acculturation from the Mataraman culture.

Wengker came from the words wewengkon angker (a mysterious place). As reflected from the etymology of the word “Wengker”, this place is known as sacred. There are many ancestral traditions which include mystical-magical rituals, which are still practiced by some of the Panaragan subethnicity until now. These traditions include larungan sesaji, bedhol pusaka, and kirab pusaka Ponorogo, which are practiced on the night of the first of every Sura (the first month in the Javanese calendar).

These traditions are continually practiced generation to generation by the Ponorogo people. The discussion on Ponorogo people cannot be separated from the customary leader, who is called the warok. Warok has a high social status in society. This social status makes a warok become respected. Thus, it is not strange how the warok has the role as the patron and protector of the society. If viewed from the meaning of the warok term, no clear terminology has been found up to now. According to Poerwawijaya warok came from the word war’I or wirangi (in Javanese language), which means wis pana, wis mangerti banget marang agal alus lahir batin, tumindake mung kangge tetulung marang liyan.

The warok is a central character in the art of reyog. His closeness with the spiritual word makes the warok identical with the mystical and magical world. It is believed that a warok must master reh kamusankan
sejati, which is the ‘path to true humanity’. It is obtained through the attitude of concern, fasting, penance, and even meditation. Because the warok has magical power, it is not seldom that people ask them for help regarding traditional healing. At least, there are two types of healings carried out by the warok, which are the non-physical healing and the traditional physical healing.

Examples of the non-physical healing are the action of healing diseases caused by ghosts such as witchcraft, trance, exorcism, etc. The warok also heals physical diseases such as sores, stomach ache, colds, etc. The two types of disease are healed through different treatments. The non-physical diseases are healed through rituals and mantras. Then, the physical diseases are healed by using traditional herbs or other media, depending on the type of disease.

Based on the two types of healing – which are the healing of the physical and the non-physical diseases – there is a similar view of the Panaragan people, both for the traditional and the modern healing. For example, in the modern view, stomach aches are believed to be caused by unhygienic foods, flus, the minimal condition of the immune system, or the unfriendly weather. It may also be caused by the violation of prohibitions determined by the ancestors, such as the consumption of certain foods and drinks which are prohibited as they endanger the body.

The description above, it becomes interesting to analyze the local wisdom of the Panaragan Java subethnicity in the field of health, with the case study of the Panaragan warok, in the case of their method of healing, their mix traditional medicine, supernatural prayers, or the combination of these things which are applied in the society.

**Method**

This research uses the method of qualitative research approach, as it is naturalistic. The reality is plural, heterogenous, and holistic, thus it cannot be measured or separated fragmentally. The researcher and the research objects are interrelated. They are inseparable, as the interact with each other to reveal the analyzed worldview. In the context of traditional healing, the effort to understand the local wisdom within traditional healings are not limited to what is visible. Yet, it tries to reveal what is hidden, so that we may see the ideology, the understanding, and the world view of the warok from Ponorogo.

This is a descriptive research, as it describes and explains the traditional healing by the Panaragan subethnicity. It tries to reveal the phenomenon of local wisdom. The descriptive method uses the ethnographic method, which has the characteristics of holistic, integrative, and which has thick description. The qualitative analysis is aimed to obtain the native’s point of view. There are two types of data in the qualitative method, which are the primary and the secondary data.

This research uses two main methods to obtain and to present the data. The first is observation. Second, the researcher carries out an interview. The type of interview used is the in-depth interview. Third, the researcher carries out a document analysis by collecting supporting documents such as journals and books. The analysis is created by study and development. This research uses a criterion-based sampling technique to obtain purposive samples to accommodate all possible data in the research field. Thus, there are no deviant cases or those which are forced to be part of a certain category. The data analysis technique is carried out through these steps: Domain analysis, Taxonomy analysis, Componential analysis and Cultural theme analysis.

**Results**

The health wisdom is the method carried out by the society in solving cases related to health problems, both which are physical and non-physical wisely. In the Panaragan Java subethnicity, especially in the art of reyog Ponorogo, there is the classification of three important domains related to health. These domains are the medical profession wisdom, medicine wisdom, and treatment wisdom. These three domains are inseparable as they are integral. This health wisdom is contained in several kinds of wisdoms, which are classified by the writer as follows:

**a. Medical profession wisdom**

This wisdom of medical profession is related to the skills of the Panaragan Java subethninc people in solving problems of various diseases. This profession is based on the warok’s type of expertise in handling some problems related to health. This expertise is ordained to the people because of their capabilities in handling various health problems. For example, there is the type of warok who is an expert in the field of health. Generally, they are
called *dukun* (shaman). This *dukun* usually heals general diseases, both which are physical and non-physical.

There are also *dukun* who have a specialization in healing diseases of muscles, such as sprain, twisted muscles, and broken bones. People call them the massage *dukun*. For example, Mr. M and Mr. IR. Mr. M is known as a *dukun* who can heal the disease of madness, but his specialization has shifted into massaging, even though he still heals people with the disease of madness. Meanwhile, a *warok* who has specialization for children are called the baby *dukun*. They usually handle diseases suffered by babies, as done by Mr. S and Mr. M. They handle diseases including *sawanen* (a condition where children are thought to be disturbed by ghosts), and they massage babies, and they also help anything to do with babies.

b. Medicine wisdom

The medicine wisdom is the method in handling types of diseases by making use of traditional herbal medicine. The traditional medicine used are from plants, including ginger, galangal, turmeric, *laos*. The usage of the herbal medicine is according to the type of disease, as each herbal medicine has its own use and function. Apart from herbal medicine, they also use other traditional medicine such as water and salt, which are believed to be able to neutralize negative aura in the human body. The wisdom of these medicine is according to the type of disease; thus, the type of medicine is based on the disease. For example, the type of medicine used by *warok* in the Reyog art of Ponorogo are as follows:

1) Achy rheumatic potion

Red onions, ginger, clove oil, and water are mixed together. They are mashed up by grinding. Then, this potion is smeared on the painful parts of the body. The red onions and the ginger are believed to be able to warm up the body, and that it may increase the blood flow. The achy rheumatic is usually suffered by the Reyog players, and they are usually healed by using that traditional method.

2) Head ache potion

Bitter coffee or eucalyptus leaves. Bitter coffee is believed to be able to ease the tensed-up nervous system. Then, the sufferer is advised to sniff ground cajuput leaves are believed to be able to ease the tensed-up nervous system and to ease the mind. Usually, in the Reyog art group, there are people who cannot take the aroma of the offerings, so they become dizzy. These simple methods may ease their dizziness.

3) Fever potion

(The documentary photo of Mr. P, with Coffee, Turmeric, and Water placed on a tray in front of Reyog)

The turmeric is one of the elements in the Reyog offering, with water and coffee. The philosophy of turmeric is *nirajim*, the water is clarity of thought, and coffee gives off pleasant aroma.

4) Potion to treat *sawanen*

*Dlingo* leaves are a type of soft-stemmed plant which has an aroma similar to garlic. Part of the society believes the myth that these leaves may shoo away genies, as the smell is not liked by Satan.

5) Potion for possessed people

Water and salt are believed to heal those who are possessed by evil spirits. This non-physical disease comes from the disturbance of genies, and it can be healed by using salt and water. Water is believed to be able to neutralize Satan’s disturbance. Satan is made of fire, and fire can be extinguished by water. Salt comes from sea water which has the philosophy that the sea is never dirty, as all rubbish that comes to the sea will be pushed to the shores.

c. Treatment wisdom

The treatment wisdom is the manner practiced to heal diseases suffered by a person so he/she may be healthy as before. The players of the Reyog art, which is a form of dance which uses physical moves, usually experience pains in the joints, muscles, and achy rheumatic. These types of conditions are caused by lack of warming up. It may also be caused of lack of practice of unpredictable
accident.

The type of health condition is then treated by urut (which literally means to order). The lexical meaning is to massage certain parts of the body which is in pain, such as strain, twisted muscles, pains, pinched nerves, and bruises. Before massaging the painful parts of the body, the warok will chant a mantra as follows:

Assalamualaikum Kakang kawah, adi ari ari, kiki kipu nini kipu, bahu tengen bahu kiwa, awalane srina sewengini sedulure putuku nek ngembani panjang umur rejekine anteng, eyang eyang singgungkuli bapa biyong, sing ngayomi anak putuku paringi sehat, lara encok loro ayu lara dengen, oleho sak njobone sumsum aja sak njerone belung sri tolah nek ngembani panjang umur rejekine anteng, eyang eyang singngungkuli bapa biyong, sing ngayomi anak putuku paringi sehat, lara encok loro ayu lara dengen, oleho sak njobone sumsum aja sak njerone belung sri tolah nek ngembani panjang umur rejekine anteng, eyang eyang singngungkuli bapa biyong, sing ngayomi anak putuku paringi sehat, lara encok loro ayu lara dengen, oleho sak njobone sumsum aja sak njerone belung sri tolah wasalamualaikum (Mr. M).

After praying, the warok will massage the painful parts of the body. He will smear red onions and oil on the body parts while massaging it, so that the body will feel warm. Another condition which appears to those around the reyog art show is sawanen. It is usually suffered by little kids after having watched the show. If there is a little kid who experienced sawanen, thus the warok will chant the following prayer.

Kakang kawah adi ari ari sedulure batur aja mincok ning manungsa panggonamu ning....ning ara ara asal balik neng ara ara, asale ning dwuwr balik neng dwuwr, asale ngisor balika ngisor aja mincok ning drema manungsa (Mr. M)

The warok will pray by suwuk while kissing the little child’s forehead. Usually, people can tell if a kid experienced sawanen by seeing the indicators. Usually the kid with sawanen will often cry and throw tantrums.

The reyog art show, there are usually spectators who experienced disturbance from the mystical realm. They may be possessed by evil spirits. The symptoms of this non-physical disease are the sufferer having a blank mind, daydreaming, or screaming unconsciously. The disease may be healed by reading the following mantra.

“Dhedhemit podo morat marit, Jin setan mawut ra karu-karuan, Kaya watu bledug dadi awu, kun faya kun kersaning Allah, Laillahaillaallah Muhamadar Rosullullah” (Mr. P).

After reading that mantra, the warok will smear salted water to the possessed person’s face or forehead. The water or toyo petak which has been given the mantra may absorb the positive energy of that mantra. Thus, it may help neutralize the negative effects in the person’s body.

Discussion

The traditions which exist in the society is the traditional healing. The society’s capability in mixing potions and also medicine and potions which may heal diseases are traditions from the previous generations. Based on the results of the research, there are local wisdoms in the aspect of health among the Panaragan Java subethnicity. This wisdom includes the wisdom of the medical profession, wisdom of medicine, and wisdom of healing.

The skills in the field of traditional health are obtained from the previous generation. Indonesians have long known and used plants with medical properties as one of the efforts to solve health problems. The use of natural products has been used by the ancestors since centuries ago. The evidence is that people still believe in the effects of herbal medicine to solve health problems.

The ability to mixing medical plants are usually followed with the ability to manage the mind, as done by the warok of Ponorogo. They believe that sometimes, diseases are not caused by visible things. They may also be caused by invisible things which come discreetly. Thus, the medicine is also from non-physical things. As carried out by warok P, who used the supernatural therapy to heal the diseases of someone being possessed by evil spirits or sawanen, by giving water with mantra.

This is in accordance with the opinions of Foster and Anderson. They stated that some parts of the society still believed that the source of some diseases are from the disturbance of invisible creatures. Further, they stated that this phenomenon is called ethnomedicine. Ethnomedicine is a practice regarding diseases which is a result of an original culture. It is explicit and does not come from modern medical science.

Conclusion

The conclusion of the health wisdom in traditional healing by the Panaragan Java subethnicity with the case study of warok Ponorogo are as follows. First, there is a wisdom of the healing profession, where someone has
the profession as a traditional healer. This profession is adapted with different specific abilities, such as child dukun, baby dukun, and general dukun. Second, the medicine wisdom, which is medicine which comes from natural plants, such as dlingo leaves, turmeric, coffee, and cengkeng. The third is the healing wisdom, which are all methods taken to heal a sick person, such as massage, blows, and smearing potions to the hurt body parts.

Conflict of Interest : No

Ethical Clearance : Yes

Source of Founding : Authors

References

Correlation between Coenzyme Q10 Level Long-Term Steroid Inhalation in Patients with Bronchial Asthma

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Abstract

Background: The main therapy of asthma is inhaled steroids and is often used for long periods of time. Coenzyme Q10 is a potent antioxidant produced largely by mitochondria. Treatment of long-term oral steroids can cause mitochondrial damage which lowers the level of coenzyme Q10.

Objective: To analyze the relationship between coenzyme Q10 levels and long-term steroid inhalation in asthma patients.

Method: The study was conducted at Asthma Unit/COPD of Dr. Soetomo General Hospital Surabaya Indonesia, Pulmonology Unit of Dr. M. Soewandhie General Hospital Surabaya Indonesia, and Pulmonology Unit of Universitas Airlangga Hospital Surabaya Indonesia. We measured coenzyme Q10 levels and the duration of inhaled steroid use in the subjects. The data were processed using computer statistics program. The correlation between coenzyme Q10 level and long-term steroid inhalation was analyzed using Pearson correlation test (p < 0.05).

Result: Coenzyme Q10 levels in all samples were normal and increasing. There was no low coenzyme Q10 level found in all samples. The result of Pearson’s correlation test between coenzyme Q10 level with long-term steroid inhalation showed r = -0.037; p = 0.848 (p> 0.05).

Conclusion: There was no correlation between coenzyme Q10 level and long-term steroid inhalation found in this study.

Keywords: Coenzyme Q10, Asthma, long-term steroid inhalation

Introduction

Asthma is a heterogeneous disease characterized by chronic airway inflammation ¹. To this day, asthma is still a problem in both developing and developed countries. Symptoms of asthma vary from the lightest, which do not disrupt activities, to the more permanent one that disrupt activities, including daily activities ²³. Approximately 300 million people around the world suffer from asthma. The average prevalence of asthma incidence in the world ranges from 1% to 18%. In Indonesia, according to basic health research data in 2007, the asthma prevalence was 4%. Basic health research data in 2013 showed that the prevalence of asthma at all ages was 4.5%. The highest prevalence was in Central Sulawesi (6.9%), followed by East Nusa Tenggara (7.3%) and Yogyakarta (6.9%). Asthma is included in the top ten causes of morbidity and mortality in Indonesia. The household health survey (SKRT) in 1986 showed that asthma is ranked fifth out of 10 causes of morbidity along with chronic bronchitis and emphysema ⁴.
Chronic inflammation in asthma leads to an imbalance of the pulmonary antioxidant system. This increases free radicals that will impact the hyperspecific and obstruction of the airway resulting in complaints triggering the formation of oxidant enzymes Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, mieloperoksidase, eosinophil peroxidase and NO synthase. ROS is released into the airway by the inflammatory cells that occur in the lungs.

Blood antioxidant levels, especially coenzyme Q10 are low in asthma patients compared to healthy people. This is due to the occurrence of oxygen-free radical processes derived from the lipid membranes in cells that accumulate in the bronchial mucosa. Coenzyme Q10 is a potential antioxidant that protects the body from free radicals and helps the supply of vitamin E (antioxidant and major cell membranes). Coenzyme Q10 plus tocopherol and vitamin C can reduce the need for steroid dose of bronchial asthma patients. This suggests a decrease in the inflammatory process of antioxidant supplementation. International guidelines recommend assessment of an APE or spirometry series to confirm the diagnosis of asthma, but spirometry can not provide clear information about the state or degree of airway inflammation at that time.

Low blood coenzyme levels in bronchial asthma and in individuals receiving long-term oral steroid therapy. Oral administration of prednisolone steroid for a long periods can cause mitochondrial cell damage. Mitochondrial damage can cause an oxidant-antioxidant imbalance. Coenzyme Q10 is a powerful antioxidant in mitochondria. Mitochondrial damage will cause deficiency of coenzyme Q10 causing free radicals and aggravate inflammation. Long-term administration of prednisolone oral steroids can affect mitochondrial damage.

The main therapy of bronchial asthma is anti-inflammatory inhaled steroid. Many people with asthma require inhaled steroid therapy in a long time to be able to control their asthma. Inhaled steroids can affect the systemic if used continuously and in a long time, causing it to affect blood coenzyme Q10 levels. To date, there has been no study examining the correlation between coenzyme Q10 levels and long-term inhaled steroids in patients with bronchial asthma. Therefore, we are interested in conducting this study to examine the correlation between coenzyme Q10 and long-term inhaled steroids in people with bronchial asthma.

**Method**

The subject of this study consisted of patients with bronchial asthma that met the inclusion and exclusion criteria. The inclusion criteria included patients with stable asthma treated in pulmonology and asthma unit/COPD on a regular basis, aged of above 21 and treated with inhaled steroid in a long period of time. The exclusion criteria excluded patients with asthma exacerbations attack, patients with other respiratory disorders (pneumonia, COPD, or other chronic respiratory diseases), patients with systemic disorders (heart disorders, parkinsonism, malignancy, diabetes, hypertension, HIV, dyslipidemia and renal failure), patients consuming certain medication (class of statin, beta blocker, diuretic, glucophage, haloperidol), and patients consuming ubiquinone preparations on a regular basis. Subjects that were willing to be enrolled in this study were asked to fill the informed consent.

This is a cross-sectional observational analytic study conducted at Asthma Unit/COPD of Dr. Soetomo Teaching Hospital Surabaya Indonesia, pulmonology unit of Dr. M. Soewandhie General Hospital Surabaya Indonesia and Universitas Airlangga Hospital Surabaya Indonesia. The amount of subjects was determined by identifying them based on the criteria and identifying their use of inhaled steroid for 30 months or more according to anamnesis, medical records, and the records of medication taken. We obtained 29 subjects.

We conducted this study by collecting the data of the subjects’ characteristics including age, body weight, height, BMI, FEV1 pred, FEV1/FVC pred, ACT score, long-term use of steroids, coenzyme Q10, sex, occupation, controller, reliever, asthma degree, type of asthma. Measurement of serum coenzyme Q10 level using Agilent tool with HPHPLC method.

The data were collected based on data obtained during the study that met the subject criteria. The data obtained were processed manually and were presented in the form of tables and graphs. The data were processed using computer statistics program SPSS version 23.0 (SPSS, Inc., Chicago, IL). Prior to statistical test, shapiro wilk test were performed. If the test result of shapiro wilk was p<0.05 then statistical test of coenzyme Q10 and long-term steroid inhalation was analyzed using spearman rank test (p <0.05). If otherwise, the statistical
The test used was Pearson correlation test.

**Result**

**Demographic Data**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean±SD (n=29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>50.55±7.52</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>58.31±11.25</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>154.69±6.51</td>
</tr>
<tr>
<td>BMI</td>
<td>24.34±4.26</td>
</tr>
<tr>
<td>FEV1 pred (%)</td>
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</tr>
<tr>
<td>FEV1/FVC pred (%)</td>
<td>64.48±13.67</td>
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<tr>
<td>ACT Score</td>
<td>19.48±4.75</td>
</tr>
<tr>
<td>Steroid Period (Month)</td>
<td>44.38±11.55</td>
</tr>
<tr>
<td>Coenzyme Q10</td>
<td>1.38±0.54</td>
</tr>
</tbody>
</table>

The result of identification of 29 subjects showed that the mean of the subjects’ age was 50.55 ± 7.52 years old, with the youngest subject being 32 years old and the oldest 64. All subjects who were willing to be involved in this study were over 21 years old. The mean of the subjects’ weight was 58.31 ± 11.25 kg, while for the height was 154.69 ± 6.51 cm. According to the value of body height and weight, the obtained Body mass index (BMI) value was 24.34 ± 4.26 kg/m2 with BMI value range of 17.00-32.00 kg/m2. The result of asthma control test score (ACT) subject showed the score of 19.48 ± 4.75 (Table 1).

<table>
<thead>
<tr>
<th>Variable</th>
<th>categori</th>
<th>% (n=29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>6.90</td>
</tr>
<tr>
<td></td>
<td>Female</td>
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</tr>
<tr>
<td>Occupation</td>
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</tr>
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<td></td>
<td>Teacher</td>
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<tr>
<td></td>
<td>Taylor</td>
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<td></td>
<td>Private Employee</td>
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<td></td>
<td>Navy Soldiers</td>
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<tr>
<td>Controller</td>
<td>seretide®</td>
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</tr>
<tr>
<td></td>
<td>symbicort®</td>
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<tr>
<td>Reliever</td>
<td>berotec®</td>
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<tr>
<td></td>
<td>ventolin®</td>
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<tr>
<td>The degree of Asthma</td>
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<td></td>
<td>Moderate</td>
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<td>Partly Controlled</td>
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<td></td>
<td>Controlled</td>
<td>20.70</td>
</tr>
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</table>
The next subject characteristics were based on the frequency distribution of subject demographic data. The majority of subjects were female (93.10%). Most subjects also used controllers with Symbicort type (62.10%) and reliever with Berotec type (82.80%). The majority of subjects had asthma in the moderate category (93.10%) and most subjects had uncontrolled asthma (41.40%; table 2). Subjects used steroid medications for $44.38 \pm 11.55$ months. The shortest span was 31 months and the longest was 72 months. The mean of coenzyme Q10 profile in the sample was $1.38 \pm 0.54$ mg/L with the lowest value being 0.51 mg/L and the highest 2.53 mg/L (Table 1).

**Correlation between Coenzyme Q10 Long Steroid Inhalation Use Period**

According to the result of Pearson correlation test between coenzyme Q10 with long-term steroid inhalation usage showed $r = -0.037$ and $p = 0.848$, indicating no significant relationship between coenzyme Q10 and long-term steroid inhalation. Pearson correlation test result showed that coenzyme Q10 with long seretide® inhalation usage was $r = 0.380$ and $p = 0.912$ which means that there was no significant relationship between coenzyme Q10 and long seretide® inhalation. Pearson correlation test results showed that coenzyme Q10 with long-term symbicort inhalation usage was $r = -0.194$ and $p = 0.441$, suggesting no significant relationship between coenzyme Q10 and long-term symbicort inhalation.

Figure 1 shows that the data spreads irregularly which means there is no significant relationship between the two variables. However, the results of the Pearson correlation coefficient with minus value and the tendency of the scatter plot image indicate that the longer the use of inhaled steroids increase the tendency of lower coenzyme Q10.

**Discussion**

To this day there is no literature or research that assess coenzyme Q10 on the use of long-term inhaled steroids. Coenzyme Q10 levels are low on the use of long-term oral steroids. The cause of low levels of coenzyme Q10 is mitochondrial damage. The damaged mitochondria can be established definitively through tissue biopsy but are too invasive. The non-invasive way used to assess mitochondrial damage is by measuring the levels of coenzyme Q10 which is a natural antioxidant synthesized in mitochondria $^{11,12}$.

In this study there is no low coenzyme Q10 level and therefore we expected the function and structure of the mitochondria in this study sample are within normal limits. Mitochondrial damage can be caused by various diseases including heart problems, Parkinson’s, malignancy, diabetes, hypertension, HIV, dyslipidemia and kidney failure and patients with those problem have been excluded from this study. We have also excluded drugs that can cause low levels of coenzyme Q10 including beta blockers, diuretics, statins, glipizide and haloperidol and asthma sufferers who consume ubiquinone supplementation have also been excluded. By conducting this study we hope to understand whether or not the use of steroids in the long term can cause low levels of coenzyme Q10 and then to use the knowledge to improve the management of bronchial asthma.

The systemic side-effects that are expected for long-term use of steroid inhalation are not proven. This may be due to the duration of inhaled steroid use that was not long enough. In this study we used a 30-month baseline, following oral prednisolone steroidal studies given for 16, 24, and 30 months $^9$. With the assumption that the longest period was 30 months, the benchmark duration of steroid inhalation in this study is then more than 30 months. Systemic side effects of steroid inhalation use in asthma were related to adrenal gland disorders, osteoporosis and skin thickening. A study discusses high doses of inhaled steroids that may have adverse systemic effects. In this study we use steroid dose in accordance with the recommended GINA based on step-up and step-down therapy theory, causing the systemic side effects to be suppressed $^{12}$. Systemic effects that can cause mitochondrial damage do not occur and therefore the coenzyme Q10 levels are not low.

The use of long-term symbicort® steroids showed the trend of lower coenzyme Q10 when compared to long-term seretide® use. Budesonid turbuhaler deposited in the lungs was 2.2 times larger than the MDI fluticasone and 3.4 times larger than the fluticasone discus. The systemic availability of budesonid turbuhaler was also higher than that of fluticasone discus and MDI $^{13}$. Budesonid turbuhaler that was 4 times larger was deposited in the lung and spread to systemic when compared with fluticasone discus $^{14}$. Coenzyme Q10 is affected by its endogenous production, its use by the body’s cells and the intake of food. Endogenous production is strongly influenced by cell mitochondria. Its use is strongly influenced by oxidative stress levels.
Some food and beverage products containing coenzyme Q10 such as meat (deer, pig’s heart, beef heart, beef liver, pork liver, beef, pork, chicken and egg), dietary fat (repeseed oil, tuna, and grilled frozen fish), cereals (wholemeal bread and wholemeal bread), vegetables (whole grains, cauliflower, nuts, and carrots), fruit (blackcurrant, lingonberry, strawberries, oranges, apples), and some dairy products (yogurt, emental cheese, edam cheese, and milk with 1.5% fat)\textsuperscript{15}.

Bronchial asthma has varying levels of oxidative stress, causing the need for coenzyme Q10 to increase. This can be accomplished from endogenous formation derived from mitochondria and adequate nutritional intake. Endogenous synthesis is more influential on the adequacy of coenzyme Q10 levels compared with exogenous nutrient intake. The normal requirement of coenzyme Q10 is 30-150 mg per day and can increase as the needs increase. When the mitochondria as a coenzyme producer of Q10 is damaged, the coenzyme content of Q10 will be low\textsuperscript{15}. Some levels of coenzyme Q10 are found to be higher than the normal range. Coenzyme Q10 levels may increase with increasing BMI\textsuperscript{48}. This is due to the fat-soluble nature of coenzyme Q10\textsuperscript{11,12}.

**Conclusion**

There was no correlation between coenzyme Q10 and long-term steroid inhalation.

**Ethical Clearance:** This research involves participants in the process using a questionnaire that was accordant with the ethical research principle based on the regulation of research ethic regulation. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, non-maleficence, and justice.

**Conflict of Interest:** The authors have not found any conflict of interest related to this research so far.

**Source of Funding:** All of the cost and fees related with this research are paid by the authors only with no sponsorship nor external funds.

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**Reference**


Association between Fear of Falling, Balance and Functional Mobility in the Elderly

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Abstract

Background: Falling is the main problem that causes fear of falling in the elderly. Impaired balance is an important factor that causes falling. Falls Efficacy Scale-International (FES-I) is a tool to measure fear of falling, while balance condition can be measured using Biodex Balance System-SD (BBS-SD). Moreover, Timed up and Go Test (TUG) is a tool to measure functional mobility. The research aimed to determine correlation between fear of falling, balance and functional mobility in the elderly.

Methods: The research was conducted in 39 elderly subjects at Outpatient Medical Rehabilitation Clinic of Dr. Soetomo General Hospital, Surabaya, Indonesia, who were measured using FES-I, BBS-SD and TUG.

Results: The FES-I and BBS-SD probability values showed no correlation between fear of falling and balance, with p=0.064 (p>0.05). Moreover, the FES-I and TUG probability values also showed no correlation between fear of falling and functional mobility, with p=0.251 (p>0.05).

Conclusion: There was no correlation between fear of falling, balance and functional mobility in the elderly at Outpatient Medical Rehabilitation Clinic of Dr. Soetomo General Hospital, Surabaya, Indonesia.

Keywords: balance, elderly, fear of falling, functional mobility

Introduction

The aging process is not a disease or a disability condition, even though most elderly suffer from functional decline that is often caused by various chronic diseases that generally accompany the aging process. The aging process is the sum of all changes that occur by the time. These changes become the cause or are strongly related with increased body vulnerability to stimuli from within and outside the body. The real example of this aging process is a gradually limited body function capacity and reduced speed capacity. There are some peculiarities of elderly patients. Those peculiarities include more diseases, unspecified disease symptoms, reduced physiological reserves, drug sensitivity and complex biopsychosocial problems.

Falling is the most common incidence experienced by elderly, and it may result in morbidity and mortality. Falling occurs when a person suddenly loses balance that eventually makes a contact with floor or other surfaces. There are many factors can cause falling in the elderly, one of which is fear of falling. Fear of falling commonly occurs in the elderly, and it correlates with decreased balance, anxiety, depression and falling history. A community-based epidemiology study reported 32-83% incidence rates in the elderly, and 33-46% of the elderly who did not fall were afraid of falling. In the elderly who experienced fear of falling, almost 70% of them avoided activities because of fear of falling. In some cases, elderly people confine themselves at home because of the fear. The activity limitation is actually a risk factor of falling because it can lead to muscle atrophy, decreased condition and balance.

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addition, this limitation cause social isolation, decreased functional activity and life quality. This research was conducted to analyze correlation between fear of falling, balance and functional mobility in the elderly. Fear of falling can be measured using Falls Efficacy Scale-International (FES-I) questionnaire. Balance and functional mobility can be measured using Biodex Balance System-SD (BBS-SD) and Timed up and Go Test (TUG) respectively.

**Method**

This research is an observational analytic study using cross sectional study design. The study was conducted in Medical Rehabilitation Unit of Dr. Soetomo General Hospital and Universitas Airlangga Hospital, Surabaya, Indonesia, from January-March 2014. The samples met inclusion criteria (males and females aged 60-89, can walk at least 6 meters without aids and can obey simple commands) and had willingness to participate in the research by signing informed consent. The study protocol was approved by the ethics committees of Dr. Soetomo Teaching Hospital (Surabaya, Indonesia).

There were 39 elderly subjects met inclusion criteria and not included in exclusion criteria. Functional mobility was measured using Timed Up and Go test, followed by a balance test using Biodex Balance System-SD. The data analysis was conducted using SPSS 17.0 software (SPSS, Inc., Chicago, IL). Afterward, the data were analyzed using Spearman’s correlation test since the data were ordinal.

**Result**

Demographic and clinical characteristics were presented in Table 1, with 39 subjects. The average subjects’ age was 67.03±6.32, with the youngest and oldest age were 60 and 80 respectively. There were 18 subjects aged 60-64 (46.15%), 7 subjects aged 65-69 (17.95%), 8 subjects aged 70-74 (20.51%), 4 subjects aged 75-79 (10.26%) and 2 subjects aged 80-84 (5.13%). Moreover, there were 8 male subjects (20.5%) and 31 female subjects (79.5%).

The results of fear of falling assessment using FES-I were less varied. The average FES-I value was 17.62±3.25, with the lowest value of 16 and the highest value of 35. The value ranged from 16 to 64, in which 16 indicated mild fear of falling and 64 showed severe fear of falling when performing 16 activities. The higher the FES-I value, the greater the fear of falling. The FES-I assessment results were shown in Figure 5.1. The study found 34 subjects with FES-I value of 16-19 (87.2%), 4 subjects with FES-I value of 20-27 (10.3%) and 1 subject with FES-I value of 28-64 (2.6%).

The results of balance assessment using Biodex Balance System-SD were varied. The average stability index was 2.36±1.36, with the lowest value of 0.60 and the highest value of 4.90. The higher the stability index, the balance gets worse thus the risk of falling is greater.

The results of functional mobility using TUG test were greatly varied. The average TUG time test was 11.33±2.73, with the lowest value of 7.90 seconds and the highest value of 20.80 seconds. The larger the TUG time, the functional mobility capacity gets worse. The assessment data were presented in Table 2.

The results of statistical test using Spearman’s correlation test showed no correlation between fear of falling, balance and functional mobility, with r=0.300 and p=0.064 (Table 3). Fear of falling did not correlate significantly with TUG, with r=0.188 and p=0.251 (significant correlation if p<0.05; Table 3)

<table>
<thead>
<tr>
<th>Table 1 Subjects’ characteristics</th>
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<tbody>
<tr>
<td>Variables</td>
</tr>
<tr>
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</tr>
<tr>
<td>60-64</td>
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<tr>
<td>65-69</td>
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<tr>
<td>70-74</td>
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<td>75-79</td>
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<td>80-84</td>
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<tr>
<td>Sex</td>
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<tr>
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<tr>
<td>Female</td>
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</table>

<table>
<thead>
<tr>
<th>Table 2 FES-I, IS and TUG assessments</th>
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</thead>
<tbody>
<tr>
<td>Variables</td>
</tr>
<tr>
<td>FES-I</td>
</tr>
<tr>
<td>IS</td>
</tr>
<tr>
<td>TUG</td>
</tr>
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</table>
Table 3 Correlation between FES-I and Age, IS and TUG

<table>
<thead>
<tr>
<th></th>
<th>FES-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>r p</td>
</tr>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td>0.337</td>
<td>0.036 39</td>
</tr>
<tr>
<td>IS</td>
<td>r p</td>
</tr>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td>0.300</td>
<td>0.064 39</td>
</tr>
<tr>
<td>TUG</td>
<td>r p</td>
</tr>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td>0.188</td>
<td>0.251 39</td>
</tr>
</tbody>
</table>

Annotation:

r: correlation coefficient

p: p shows probability or significance level. Significant if p<0.05.

Discussion

The subjects’ average age showed fewer subjects in the age group of elder patients. This finding correlated with average life expectancy in Indonesia (70.7 years), thus the number of elderly in Indonesia decreased with age. The subject distribution showed varied numbers of elderly subjects by age. The ratio of male to female subjects was quite far (1:3), with 8 male subjects (20.5%) and 31 female subjects (79.5%).

Fear of falling was measured using FES-I as it has good psychometric elements and it was utilized to measure fear of falling in some studies. FES-I has been tested for validity after being translated into Greek. The average results of fear of falling assessment using FES-I was 17.62±3.25, with the lowest value of 16 and the highest value of 35. The value ranged from 16 to 64, in which 16 indicated mild fear of falling and 64 showed severe fear of falling when performing 16 activities. The higher the FES-I value, the greater the fear of falling. 34 subjects (87.2%) had mild fear of falling, thus they felt very unlikely to fall when performing 16 activities. There were 4 subjects (10.3%) with moderate fear of falling and 1 subject (2.6%) with severe fear of falling.

The research found a significant correlation between fear of falling and age (p=0.036). This finding was consistent with the previous study which reported that fear of falling commonly occurred in females compared to males, and it usually increased with age.

The results of balance assessment using Biodex Balance System-SD were varied. The average stability index was 2.36±1.36, with the lowest value of 0.60 and the highest value of 4.90. The higher the stability index, the balance gets worse thus the risk of falling is greater. There were 10 subjects (25.64%) having a stability index above normal, thus they had less balance which resulted in a greater risk of falling. Risk of falling in the study was found in 1 out of every 4 subjects, with ratio 1:3. If the subjects represented elderly in general, then the risk of falling in the elderly was quite large.

Functional mobility is a term used to describe balance condition and walking activity in daily life (such as sit, stand, walk, turn around). TUG test is a commonly used balance test to examine functional mobility in the elderly. The results of functional mobility assessment using TUG test were greatly varied. The average TUG time was 11.33±2.73, with the lowest value of 7.90 seconds and the highest value of 20.80 seconds. The larger the TUG time, the functional mobility capacity gets worse. There were 9 subjects (23.08%) having TUG time above normal, thus they had less functional mobility capacity (Figure 5.3). This was resulted from walking pattern changes which was adjusted with balance, including short and slow steps. Various cross sectional studies reported that increasing age affects walking pattern changes, including slow swing and wide base patterns, longer double leg support phase, decreased stride length and torso rotation.

Frank and Patla measured correlation between capability, walking speed and fear of falling in 40 elderly subjects. They found that elderly with fear of falling showed limited capability and confidence in balance. Kalsait et al reported a correlation between fear of falling and physical performance, one of which is balance. The present study found no significant correlation between fear of falling and balance (p=0.064). This finding might be resulted from a relatively small subject distribution with fear of falling (10.3% with moderate fear of falling and 2.6% with severe fear of falling).

The results showed no correlation between fear of falling and balance, as fear of falling is a psychological factor that is associated with falling in the elderly, whereas balance is a complex interaction between sensory/
afferent system (vestibular, visual and proprioceptive) and motor/efferent system (muscles, joints and soft tissues) processed in the brain in response to changes in internal (host) and external (environment) conditions. As a result, response to fear of falling is not necessarily manifested as falling since intrinsic and extrinsic factors will respond to the body to maintain balance in order to avoid falling. Thus, self-efficacy based on psychology for fear of falling is merely one factor affecting balance. In clinical practice, this condition could be used as a precaution when serving elderly. It does not mean that elderly who has no fear of falling has a good balance, thus the risk of falling may remain.

The research found no significant correlation between fear of falling and functional mobility, with p=0.251. Falling risk identification could be performed by examining elderly balance condition when moving from one place/position to another. The study used TUG test to examine functional mobility. Someone needs a good balance to move from place/position to another. As we mentioned earlier, fear of falling is a factor affecting balance, thus there are other factors affecting elderly mobility. It can be concluded that elderly with good self-efficacy does not necessarily have good mobility since age. Meanwhile, conversely, elderly with fear of falling does not necessarily have bad functional mobility as fear of falling could be overcome with good physical condition. In clinical practice, this could become a consideration when serving elderly with fear of falling. From these findings, we could provide educational/psychological support to the elderly that falling could be prevented if one has high confidence and fitness level to avoid falling and fear of falling. It was expected that physical and psychological problems affecting balance that may result in functional mobility could be addressed by performing an exercise activity.

**Conclusion**

This research shows that fear of falling measured with FES-I questionnaire did not correlate with balance measured with Biodex Balance System-SD test. Moreover, fear of falling measured with FES-I questionnaire did not correlate with functional mobility measured with Time Up and Go test.

**Authors’ contribution:** Fatmawaty Abasmay do the laboratory research, Nuniek Nugraheni writes the report of the laboratory into this paper, while Reni Hendrarati Masduchi writes the result of the laboratory research.

**Ethical Clearance:** This research involves participants in the survey using sampling method that was accordant with the ethical research principle based on the regulation of research ethic committee. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, nonmaleficence, and justice.

**Conflict of Interest:** The authors guarantee that there is no conflict of interest.

**Source of Funding:** All of the expenses related with this study are paid by the authors without any sponsorship.

**References**


Relation between the Increase of Alpha Fetoprotein Serum Level and the Size of Hepatocellular Carcinoma in Multi Slice Computed Tomography Examination

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Abstract

Background: Hepatocellular carcinoma can be diagnosed based on the result of Multi Slice Computed Tomography (MSCT) imaging only without histology result confirmation.

Objective: The aim of the study is to find out the relation between the increase of alpha fetoprotein serum level and the size of hepatocellular carcinoma in MSCT examination in Dr. Soetomo Teaching Hospital.

Methodology: This is a retrospective, observational analytic study which uses secondary data. There were 50 samples with hepatocellular carcinoma. The data were collected from the medical records of MSCT subjects and AFP (Alpha-fetoprotein) serum level result. All recorded and analyzed statistically. Results: Of all the 50 samples, 41 are male (82%) and 9 are female (18%). The ages of the samples ranged from 19 to 76 years old with the majority of 51-60 years old in 15 patients (30%). Most distribution of AFP serum level was >400 ng/ml with 29 patients (58%) and tumor size of >5 cm with 49 patients (98%). The analysis using Spearman correlation test shows the correlation value p = 0.418 (p >0.05).

Conclusion: There was no relation is found between the increase of alfa fetoprotein serum level and the size of hepatocellular carcinoma in MSCT examination.

Keywords: Hepatocellular carcinoma, Alfa fetoprotein, Multi Slice-CT, Tumor size.

Introduction

Hepatocellular carcinoma (HCC) is the most common primary malignancy of the heart ¹,². It is ranked fifth in the world following lung cancer, prostate cancer, colorectal cancer, and stomach cancer. It is also ranked as the world’s second biggest cause of death after lung cancer with the death rate of 745,000 per year ³,⁴. HCC is different from the other types of carcinoma since it is able to be diagnosed based on multi slice computed tomography (MSCT) imaging result only without histology result confirmation ⁵–⁷. Abdominal MSCT examination is a radiology examination using X-ray and computer ⁴. In hepatocellular carcinoma patients, abdominal MSCT is conducted in 4 phases: pre-contrast, arterial phase, venous phase, and delayed phase ²,⁹. Non-ionic contrast media is injected intravenously to see the hyper-vascular lesions of hepatocellular carcinoma in arterial phase and rapid wash out in venous phase ²,⁹. Therefore the researchers aimed to find out the relation between the increase of alfa fetoprotein serum level and the size of hepatocellular carcinoma in Dr. Soetomo Hospital Surabaya.

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budilaraswati1@gmail.com
Material and Method

Subject

The subject of this study is all abdominal MSCT patients of radiology installation in diagnostic center building Dr. Soetomo General Hospital Surabaya during January to December 2014 whose hepatocellular carcinoma results has never been treated and already has AFP (Alpha-fetoprotein) serum level.

Method

This research is observational analytical with retrospective study (secondary data from Medical records). This study involved 50 abdominal MSCT patients whose hepatocellular carcinoma results have never been treated and have AFP serum level. The criteria for this study is that all abdominal MSCT patients with hepatocellular carcinoma results who come for the first time and have never undergone therapy and have AFP serum level examination results.

Two variables were used in this study as research variables: alfa fetoprotein serum level and tumor size in MSCT. The data were collected from the medical records of MSCT patients with hepatocellular carcinoma in Radiology Installation, Diagnostic Center Building, Dr. Soetomo General Hospital Surabaya. The data were categorized based on the research criteria and AFP serum level result. All documentation results were gathered for data tabulation and statistical analysis later. Analysis test in this study was conducted using Spearman test. Statistical measurement was done using SPSS software. The authors have obtained ethical clearance permission from Medical Research Ethics Committee of Dr. Soetomo General Hospital.

Result

Research Subjects’ Characteristics

The result of this study showed that there were 50 abdominal MSCT patients (n = 50) with hepatocellular carcinoma results who came from the first time, had never undergone therapy, and had AFP serum level examination results in Radiology Installation of Dr. Soetomo General Hospital Surabaya in the period of January 2014 to December 2014. Of all the 50 patients, 41 were male (82%) and 9 were female (18%), all of them met the criteria, as shown in Table 1.

<table>
<thead>
<tr>
<th>Table 1. Group Distribution Based on Gender</th>
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<tbody>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>Male</td>
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<tr>
<td>Female</td>
</tr>
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<td>Total</td>
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<th>Table 2. Group Distribution Based on Age</th>
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<tbody>
<tr>
<td>Age</td>
</tr>
<tr>
<td>---------------</td>
</tr>
<tr>
<td>11 - 20 years</td>
</tr>
<tr>
<td>21 - 30 years</td>
</tr>
<tr>
<td>31 - 40 years</td>
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<tr>
<td>41 - 50 years</td>
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<tr>
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</tr>
<tr>
<td>71 - 80 years</td>
</tr>
<tr>
<td>Total</td>
</tr>
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</table>

In this study, the distribution of hepatocellular carcinoma based on AFP level are AFP level of <20 ng/ml with 11 patients (22%), 20-400 ng/ml with 10 patients (20%) and >400 ng/ml with 29 patients (58%). The most distribution is found in tumor size of >5 cm with 49 patients (98%), while tumor size of 3-5 cm has 1 patient (2%). No patient is found with tumor size of <3 cm. From the data, it can be inferred that there is a significant difference between tumor size of <3 cm and >5 cm. The age group of hepatocellular carcinoma patients in this study was ranged between 19-76 years old. The most frequency is found in the age range of 51-60 with 15 patients (30%), followed by the age range of 41-50 with 14 patients (28%) (Table 2).
Hepatocellular carcinoma characteristics based on AFP level was shown in Table 3. AFP level and divided into 3 categories. In this study, the most distributed was found in AFP level category III (>400 ng/ml) with 29 patients (58%), while category II (20-400 ng/ml) had 10 patients (20%) and category I (<20 ng/ml) had 11 patients (22%).

The result of hepatocellular carcinoma distribution data analysis based on tumor size was shown in Table 4. In this study, the most distribution was found in tumor size of >5 cm with 49 patients (98%), followed by tumor size of 3-5 cm with 1 patient (2%). No patient was found with tumor size of <3 cm.

Statistical data analysis using Spearman’s correlation statistic test Spearman Correlation Test shows the correlation value $p = 0.418$ ($p >0.05$) which indicated no relation between the increase of alfa fetoprotein level and the size of hepatocellular carcinoma in MSCT examination.

**Discussion**

In this study, there were 50 hepatocellular carcinoma patients in January to December 2014 who meet the research criteria, with sex-based distribution of 41 males (82%) and 9 females (18%) and the ratio between male and female is 4:1. This is in line with several previous studies mentioning the ratio between male and female of 4:1. In this study, the most age is 51-60, with the age range of 19-76 and the mean age of 49. This finding is in accordance with the previous study.

The number of hepatocellular carcinoma patients in this study with tumor size of <3 cm is much less (none) compared to those of with tumor size of >5 cm. This is probably because the majority of patients who come to Radiology Installation Dr. Soetomo Teaching Hospital Surabaya in the period of January to December 2014 are already in intermediate or advance stage (where the

<table>
<thead>
<tr>
<th>Table 3. Group Distribution Based on AFP Level</th>
<th>( \text{Frequency} )</th>
<th>( \text{Percentage} )</th>
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<tbody>
<tr>
<td>I</td>
<td>11</td>
<td>22%</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>20%</td>
</tr>
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<td>III</td>
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<td>58%</td>
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<th>Table 4. Group Distribution Based on Tumor Size</th>
<th>( \text{Frequency} )</th>
<th>( \text{Percentage} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>1</td>
<td>2%</td>
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<tr>
<td>III</td>
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<td>98%</td>
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<tr>
<td>Total</td>
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<th>Table 5. Spearman Test Result</th>
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<th>Approx. Sig.</th>
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<tr>
<td>Interval by interval</td>
<td>Pearson’s R</td>
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<td>Ordinal by ordinal</td>
<td>Spearman Correlation</td>
<td>-117</td>
<td>0,059</td>
<td>-818</td>
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<tr>
<td>N of Valid Cases</td>
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</tbody>
</table>
tumor size is 3-5 cm or >5 cm) when are diagnosed. Therefore, no patient is found in early stage with small tumor size or <3 cm and there are variations of time range between AFP and MSCT examination result.

In this study, tumor with high AFP might appear small in size, but there is also tumor with low AFP yet has big size. This might due to the variations of hepatocellular carcinoma tumor that do not generate AFP or generate antigen that does not react with antibody used in immunoassay. 

Conclusion

No relation between alfa fetoprotein serum level and the size of hepatocellular carcinoma in MSCT examination, p = 0.418 (p >0.05). From the result of this study which has been statistically analyzed using Spearman’s correlation statistic test (Spearman Correlation Test), the correlation value is p=0.418 (p >0,05) which indicates no relation between the increase of alfa fetoprotein serum level and the size of hepatocellular carcinoma in MSCT test.

Conflict of Interest : There is no conflict of interest

Source of Funding : This study is self-funded

Ethical Clearance : This study was approved by Ethical Commission of Medical Research, Faculty of Medicine, Universitas Airlangga.

References


Study of Gastroesophageal Reflex Disease in Adult Type II Diabetes Mellitus patients with Upper Gastrointestinal Symptoms

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Abstract

Gastrointestinal symptoms are relatively common in clinical practice in patients with type II diabetes mellitus. type II DM has been described as possible risk factor for the development of gastroesophageal reflux disease, in this study we aim to detect the prevalence of GRED in symptomatic patients with type II DM , also to see the accuracy of reflex disease questionnaire ( RDQ) in the diagnosis of GERD in patients with type II DM in relation to the esophagogastroduodenoscopy finding and to study the relation of autonomic neuropathy to the prevalence and stages of GERD in diabetic patients .

Methods: A ninety patients with upper gastrointestinal symptoms was divided in to tow groups according to whether had type II DM or not and then each group involved in a two stage process (a ) – a RDQ , ( b ) – OGD. And patients in the DM group underwent another step to detect diabetic neuropathy by a bed side clinical test .

Results: The prevalence of esophagitis in this study was higher in the DM group. RDQ had a statistical significance in detecting esophagitis in the DM group. There is no relation of autonomic neuropathy to the prevalence of GERD in diabetic patients .

Conclusion : The prevalence of GERD was high in patients with type II DM , RDQ is a sensitive tool for the diagnosis of GERD in diabetic patients , Autonomic neuropathy did not increase prevalence of esophagitis in diabetic patients .

Key words: diabetes mellitus ,gastro esophageal reflex disease , RDQ, Esophagogastroduodenoscopy, Esophagitis.

Introduction

The current concept of GERD is “symptoms or complications associated with regurgitation from the stomach and or the duodenum to the esophagus”, It is estimated that 15%-30% of the general population are affected by GERD(1,2)

GERD develops when the esophageal mucosa exposed to gastroduodenal contents for prolonged periods of time, resulting in symptoms and, in a proportion of case , esophagitis(2).

Note that some degree of gastroesophageal reflux is normal, physiologically connected with the mechanism of belching (transient LES relaxation), but esophagitis results from excessive reflux, often accompanied by impaired clearance of the refluxed gastric juice. Restricting reflux to that which is physiologically intended depends on the anatomic and physiologic integrity of the oesphagogastric junction, a complex
sphincter comprised of both the LES and the surrounding crural diaphragm. Three dominant mechanisms of esophagogastric junction incompetence are recognized: (a) transient LES relaxations (a vagovagal reflex in which LES relaxation is elicited by gastric distention), (b) LES pressure decrease, or (c) anatomic distortion of the esophagogastric junction inclusive of hiatus hernia(1,2).

Transient LES relaxations account for at least 90% of reflux in normal subjects or GERD patients without hiatus hernia, in many studies the first two mechanisms and other factors such as abdominal obesity, delayed gastric emptying, disruption of esophageal peristalsis, autonomic neuropathy, metabolic syndrome were thought to be a possible risk factors for the high prevalence of the typical GERD symptoms in patient with DM, but up to present, the pathogenesis of GERD in type II DM patients has not been fully clarified(3,4,5).

Peripheral and autonomic neuropathy especially has become a research hotspot in recent years, however, the impact of the above factors on the presence of GERD symptoms in type II DM patients is still under debate(3,6).

Patients with GERD may present with heartburn and regurgitation which are the major symptoms of GERD, somewhat less common are dysphagia and chest pain and a variety of other features has been described, and depending on this a clinical system of symptom-based diagnosis of GERD was made the so called reflex disease questionnaire RDQ(7).

RDQ comprises 6 questions assessing the frequency of heartburn, substernal chest pain, acid regurgitation, nausea, need for the medication, and trouble getting a good night sleep because of heartburn or regurgitation, per week, as shown in table 1.

### Table 1: GerdQ Questionnaire

<table>
<thead>
<tr>
<th>SYMPTOM</th>
<th>HOW MANY TIMES DOES THIS OCCUR PER WEEK?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burning feeling behind the breastbone (heartburn)</td>
<td>0</td>
</tr>
<tr>
<td>Stomach contents moving up to the throat or mouth (regurgitation)</td>
<td>0</td>
</tr>
<tr>
<td>Pain in the middle of the upper stomach area</td>
<td>3</td>
</tr>
<tr>
<td>Nausea</td>
<td>3</td>
</tr>
<tr>
<td>Trouble getting a good night's sleep because of heartburn or regurgitation</td>
<td>0</td>
</tr>
<tr>
<td>Need for over-the-counter medicine for heartburn or regurgitation (such as Tums, Rolaids, Maalox, or other antacids), in addition to the medicine your doctor prescribed</td>
<td>0</td>
</tr>
</tbody>
</table>

**NOTE:** Add the point values for each corresponding answer. Total score of 0 to 2 points = 0 percent likelihood of GERD; 3 to 7 points = 50 percent likelihood; 8 to 10 points = 79 percent likelihood; 11 to 18 points = 89 percent likelihood.

Several investigation used to evaluate patients with suspected having GERD of which endoscopy is the initial investigation of choice, it used for the diagnosis and grading of GERD and to exclude other upper gastroduodenal disease that can mimic GERD and to identify complications.

**Aim of the study**

To study the prevalence of GERD in symptomatic patients with type II DM in relation to non DM, also to
see the accuracy of RDQ in the diagnosis of GERD in patients with type II DM in relation to the OGD finding and to study the relation of autonomic neuropathy to the prevalence of GERD in diabetic patients present with upper gastrointestinal symptoms.

**Patients and Method**

This study was conducted in Alsader medical city, in Alnajaf center for gastrointestinal and hepatic diseases in which 122 adult patients referred for upper endoscopy because of upper gastrointestinal symptoms suggestive of GERD between march 2014 and march 2015 participate in this study.

Exclusion criteria were type I DM, esophageal motility disorder other than ineffective esophageal motility, patient with hiatus hernia and patient with a RDQ less than 8.

After performing the exclusion criteria a 90 patients were considered eligible for this study 31 of them were females and 59 were males.

The recruitment patients were divided into two groups according to whether had a type II DM or not, the diagnosis of type II DM based on WHO definition (Defined as fasting blood glucose >= 7 mmol/l or on glucose medication for raised blood glucose or with a history of diagnosis of diabetes.) (8)

the then every group involved in a two stage process 
(a)- Reflux disease questionnaire (RDQ) and patients with at least a minimal score of 8 had been chosen as the (RDQ) likelihood of GERD were as fellow(7):

1- A total score 0 to 2 = 0 percent likelihood
2- A total score 3 to 7 = 50 percent
3- A total score 8 to 11 = 79 percent
4- A total score 12 to 18 =89 percent.

(b)- OGD by the use of Olympus or pentax ED-3490 TK 4.2 HOYA corporation endoscopy system, which used in the diagnosis and staging of esophagitis according to Savery-Miller classification in these patients (9)

(c)- the patients with DM group underwent another step during interview by being underwent a simple clinical test to detect the diabetic autonomic neuropathy( a bedside test for the absence respiration-associated sinus arrhythmia by monitoring the pulse rate by a pulse oximeter while the patient instructed to breathe slowly and deeply at a rate of 6 breaths per minute, heart rate normally increase with inspiration and decrease with expiration, and at a respiratory rate of 6 per minute the difference between fastest and slowest heart rate is usually more than 15 per minute , a difference of 10 beats per minute or less are observed in autonomic dysfunction(10).

**Statistical Analysis**

Statistical analysis were performed using spss 16 program correlation analysis was also performed.

A P value <0.05 was considered significant.

**Results**

Ninety patients were included in this study and divided in to two groups a thirty patients in DM group and sixty patients in the non DM group.

In the non DM group 22 of them were females(36.66%) and 38 were males(63.33%) with a mean age of (46.7years±8.2).

In the DM group 9 were females (30%) and 21 were males (70%) with a mean age of (51.2 years±9.3). As shown in table 2.

<p>| Table-2 the demographic characters of both groups (DM and non DM) |</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female</td>
</tr>
<tr>
<td>DM</td>
<td>9(30%)</td>
</tr>
<tr>
<td>Non DM</td>
<td>22(36.66%)</td>
</tr>
<tr>
<td></td>
<td>Male</td>
</tr>
<tr>
<td>DM</td>
<td>21(70%)</td>
</tr>
<tr>
<td>Non DM</td>
<td>38(63.33%)</td>
</tr>
<tr>
<td>Mean age years</td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>46.7±8.2</td>
</tr>
<tr>
<td>Non DM</td>
<td>51.2±9.3</td>
</tr>
</tbody>
</table>

The prevalence of esophagitis in this study were higher in the DM group as upper OGD results showed
esophagitis in 32 patients of the non DM group (53.3%) and in 23 patients of the DM group (76.66%) with a statistical significant results (p value=0.03236) as shown in table 3.

Table 3 (the prevalence of esophagitis by OGD in both study groups)

<table>
<thead>
<tr>
<th>Group</th>
<th>Esophagitis</th>
<th>Percentage</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non DM</td>
<td>32 of 60</td>
<td>53.33%</td>
<td>0.03236*</td>
</tr>
<tr>
<td>DM</td>
<td>23 of 30</td>
<td>76.66%</td>
<td></td>
</tr>
</tbody>
</table>

In comparing the RDQ which about 80% in a score level 8-11 and OGD result in detecting esophagitis in both groups the result shows that RDQ had a statistical significance in detecting esophagitis in the DM group as shown in table 4.

Table 4 comparing QRS and OGD in detecting esophagitis in both study groups.

<table>
<thead>
<tr>
<th>Number of patients in DM group with esophagitis</th>
<th>By QRS 80%</th>
<th>By OGD 76.6%</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 of 30=80%</td>
<td>23 of 30</td>
<td>0.00244*</td>
<td></td>
</tr>
</tbody>
</table>

In the DM group the clinical bedside test for autonomic neuropathy revile an autonomic dysfunction in 16 patients of 30 (53.33%) and 14 patients show not (46.6%).

13 (81.2%) of the 16 patients with DM and autonomic dysfunction were diagnosed with esophagitis by OGD and 3 patients show no sign of esophagitis by OGD(18.7%), while in patients with DM and without autonomic dysfunction there were 10 patients of 14 (71.4%) diagnosed with osephagitis by OGD and 4 patients (28.5%) are not, so there were no statistical significance P value=0.5287 as shown in table 5.

Table 5-previlance of esophagitis in DM patients with and without autonomic dysfunction

<table>
<thead>
<tr>
<th>Patient in DM group</th>
<th>Patient with esophagitis</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>With autonomic dysfunction</td>
<td>13 of 16 (81.2%)</td>
<td>0.5287</td>
</tr>
<tr>
<td>Without autonomic dysfunction</td>
<td>10 of 14 (71.4%)</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Diabetes is a major health problem, as it is an important contributor to various other disease and its incidence still continues to rise. Gastrointestinal symptoms are relatively common in clinical practice in patients with type II DM. Type II DM has been described as possible risk factor for the development of GERD. Several studies reported that the prevalence of GERD symptoms in the type II DM was approximately 25%-40% in different populations (11,12,13), in these studies several methods used in the diagnosis of GERD includes; RDQ which had a sensitivity of 79% in the diagnosis of GERD at 8-11 score level in non DM patients, Endoscopic examination which had high
specificity but low sensitivity for the diagnosis of GERD with a sensitivity about 55% for detecting esophagitis and other complications\textsuperscript{(14,15)} and ambulatory PH monitoring which is indicated if diagnosis is unclear or surgical intervention is under considering. Although the test yields accurate and reliable information it is inconvenient for the patient and many clinicians do not have access to the appropriate instrumentation and are unable to perform this test\textsuperscript{(16)}.

In this study a combination of RDQ and OGD is used to study the prevalence of GERD in patients with type II DM as a previous study by Lemeneh Tefera, B.A., Martin Fein. \textsuperscript{(16)} shows that this combination had a high proof value 98% and high specificity 97% for the diagnosis of GERD in non DM patients, in this study we found that the prevalence of GERD in adult symptomatic patient with type II DM as diagnosed by OGD were high 76.7% comparing to non DM patients 53.3% with a P value =0.03236.

We also found that the RDQ at level score >8 had a good prediction rate for esophagitis which about 80% in symptomatic patient with type II DM as the OGD result showed esophagitis in 76.66% in patients with type II DM comparing to non DM patients in regard to the sensitivity of RDQ with a P value=0.00244. and this was constant with a study by Yu Bai, Yiqi Du, Duowu Zou, Zhendong Jin and others, that suggest that the RDQ may be used for the diagnosis of GERD\textsuperscript{(17)}.

In previous studies neuropathy were thought of as possible risk factor for the prevalence of the typical GERD symptoms in DM patients (13,20), for example, in 2008, Wang et al. \textsuperscript{(13)} reported that the prevalence of GERD symptoms was higher in patients with neuropathy than in patients without neuropathy, in this study there were no significant difference in the prevalence of esophagitis in DM patients with or without neuropathy with a P value=0.05287, this results are similar to the studies of Clouse and Lustman \textsuperscript{(21)} and Lee et al. \textsuperscript{(19)}.

In 1989, Clouse and Lustman, through analyzing 114 diabetic subjects with gastrointestinal motor dysfunction symptoms, reported that gastrointestinal symptoms occurring in diabetic patients were poorly related to neuropathic complications. In 2011, Lee et al. \textsuperscript{(19)} studied 119 patients with type II DM and found that there was no significant difference in the proportions of patients experiencing typical GERD symptoms between the two groups of type II DM with and without neuropathy.

**Conclusion**

The prevalence of GERD were high in patients with type II DM, RDQ is a sensitive tool for the diagnosis of GERD in those patients, Autonomic neuropathy did not increase prevalence of esophagitis in diabetic patients.

**Conflicts of Interest**: None of the authors have any conflicts of interest relevant to this research subject.

**Ethical clearance**: The study was conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki. The study protocol, care of patients and subject information were reviewed and approved by a local Ethic committee.

**Source of Funding**: Self

**References**

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15. American college of physician , gastroesophageal reflux Disease , Gastroenterology and Hepatology, MKSAP 16ed 2012 .


Association between Dose and Duration of Cisplatin Exposure with Sitotoksity Effect on Nasopharyngeal Carcinoma Stem Cell

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Abstract

Background: Nasopharyngeal carcinoma (NPC) is ranked 6th of malignant tumors in Indonesia, while cisplatin is an effective chemotherapy therapy but the side effects and resistance problems are two major constraints limiting its application.

Objective: To analyze the correlation of dose and duration of cisplatin exposure with cytotoxic effects on nasopharyngeal carcinoma stem cells

Methods: A true experimental laboratory in vitro with the factorial design was used in this study. The biopsy NPC tissue was cultured and processed to obtain NPC stem cells to be treated with cisplatin exposure at different doses (0.05, 0.1, 0.2, 0.4, 0.8, 1 and 2 μg) and different durations (24 and 48 hours). The number of dead cells after exposure will be calculated using a hemocytometer after 24, 48, and 72 hours of NPC stem cells free of cisplatin.

Results: Death stem cell density of NPC was mostly obtained at the exposure of 2 μg/ml cisplatin dose after 24 hours observation was 81.37%, while the smallest death cell density a dose of 0.05 μg/ml after a 72-hour observation was 21.3%. Statistical analysis with multiple regression correlation tests between the density of death cell and cisplatin dose was obtained the coefficient correlation 0.827 and value p = 0.000. The analysis of the correlation between cisplatin exposure duration and death cell was also significant with the correlation coefficient -0.357 and the value p = 0.001. The cutoff point that correlated the dose and death stem cell of NPC by 50% in both 24 and 48 hours of exposure was 1 μg (EC₅₀).

Conclusion: There was a correlation between the increased dose of cisplatin with the cytotoxicity effects on NPC stem cell.

Keywords: Nasopharyngeal carcinoma (NPC), Dose, Duration Of Exposure, Cisplatin, Cytotoxicity

Introduction

The incidence of Nasopharyngeal Carcinoma (NPC) in Indonesia is 6.2/100,000 inhabitants every year. NPC is ranked 6th of malignant tumors in humans after malignant tumors of the cervix, liver, breast, lung, and skin. According to research both national and international, it is reported that most of NPC patients (80%) come in advanced stages III and IV. During the last five decades, NPC handling is mainly in various forms of chemotherapy and radiation therapy. The damage effect caused by chemotherapy is called cytotoxicity, that most chemotherapy drugs work by interfering cell mitosis and primarily targeting cells with high cleavage rates ¹.

Cisplatin is an effective chemotherapy, but side effects and resistance problems are two major constraints limiting its application. The biochemical mechanism of cisplatin cytotoxicity includes the correlation between...
DNA and non-DNA targets which will induce death cell through apoptosis, necrosis or both. The cytotoxicity indicator used is EC50 (effective concentration 50) which is the dose/concentration of certain compounds that needed to produce a cytotoxic effect of 50% death cell in cell culture in vitro. This indicator is often used as a benchmark of eukaryotic cell cytotoxicity in culture 2.

Research that correlated the role of resistant cancer stem cells to cisplatin and the progression of many malignancies were included in ca mammae studies was reported that the presence of tumor cells were expressing the normal tumorigenic stem cell characteristics by 5.9% of the tumor cell population 3. The population of these cells significantly increased to an average of 8.8% in primary transplants that only responded partially to cisplatin, while in secondary tumor transplants, the population increased to 22.8% 4.

In vitro research on the effect of cisplatin on DNA suggests that cisplatin toxicity is affected by dose and time/dose also time-dependent. The previous research mentioned the concentration of certain cisplatin was needed to kill 90% of cancer cells. While the other studies reported that 24-hour cisplatin exposure was significantly much more cytotoxic than the first hour duration of exposure 5. Based on these descriptions, this study was conducted to reveal the cytotoxic effects of cisplatin chemotherapy drugs on NPC stem cells and the dose also duration that affect them. This approach useful for understanding how NPC stem cells process cisplatin exposure and determining the effect of the dose also the duration of cisplatin exposure resulting in cytotoxic effects on NPC stem cells 6.

**Method**

This study is experimental laboratory in vitro with the factorial design. Biopsy specimens from a patient suspected of NPC were taken in sufficient quantities, and some were sent to the Anatomical Pathology Installation Dr. Soetomo Genera hospital in formalin solution. Nasopharyngeal carcinoma stem cell culture divided into two plates 7.

After the culture procedure, stem cells nasopharynx carcinoma confirmed with CD44 + staining that divided into two plates of each containing 24 well. Each well is filled with 100,000 stem cells of nasopharyngeal carcinoma. One plate was incubated for 24 hours and another plate for 48 hours, after 24 hours exposure, NPC stem cells in one plate were cleared of cisplatin and observed at 24, 48, and 72 hours before calculation. The same procedure for 48 hours of cisplatin exposure was performed in a similar method 8. The density of the dying cell from the original mixture will be calculated according to the percentage of total number of dead NPC stem cells divided by total number of NPC stem cells (dead and alive cells) 5.

**Results**

The data shows the number of cells experiencing mortality at 24 hours cisplatin exposure and observation periods at 24, 48 and 72 hours. The number of cells that died after cisplatin administration increased along with increasing the doses (compared with controls). The pattern increase occurs with a little fluctuation were the decrease in the dose of 0.4 μg at 24 and 48 hours of observation then increase again. At 72 hours observation the decrease occurred at a dose of 0.8 μg and then the number of dead cells increased (Table 1).

Death stem cell density of NPC mostly in the 24 hours cisplatin exposure was obtained at 2 μg/ml with the post-observation time after exposure was 81.37%, while the smallest death cell density at 0.05 μg/ml dose calculated after 72 hours observation was 21.3%. The proportion of dead cells was relatively high after post-24 hour observation, while post-observation 48 and 72 hours of the relatively close coincident pattern but not as high as post-24 hour observation.

Table 2 shows a similar pattern to 24-hour cisplatin exposure that is an increase in dose-dependent death cell, there was a slight decrease in the dose of 0.1 μg, then increased again. On a 48-hour observation, death cell was high at a dose of 0.05 μg, dropping at 0.1 μg, then just increasing again at 0.4 μg. On a 72-hour observation, death cell dropped at a dose of 0.1 μg, then increased at a dose of 0.2 μg. Density stem cells of NPC mostly at 48 hours cisplatin exposure was obtained at 2 μg/ml cisplatin dose exposure with a post-observation time at 72 hours after exposure was 51.32%.

Table 2 shows that death cell density after 48 hours of cisplatin exposure tends to increase according to the observation period. The density of death cell at 48 hours of observation was relatively higher than 24 hours, while after 72 hours observation showed the highest density. From statistical analysis with multiple regression correlation tests between the density of death cell
(proportion of dead cells) and cisplatin dose was obtained the correlation coefficient 0.827 and \( p = 0.000 \). Table 1 and 2 show tendency pattern of increased death cell with higher cisplatin doses. Both of these showed that there was a significant correlation between the increased dose of cisplatin and cytotoxicity in NPC stem cells (\( p < 0.05 \)).

Data correlation of cisplatin exposure duration with death cell was obtained coefficient correlation -0.357 and \( p = 0.001 \) indicating that there was the correlation between duration of cisplatin exposure with cytotoxicity profile on NPC stem cells which also significant (\( p <0.05 \)).

### Table 1. Number and density of dead cells in post-exposure solution of 24-hour cisplatin

<table>
<thead>
<tr>
<th>Concentration (mg/ml)</th>
<th>Observation 24-hour</th>
<th>Observation 48-hour</th>
<th>Observation 72-hour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Live</td>
<td>Dead</td>
<td>Death cell density (%)</td>
</tr>
<tr>
<td>0.05</td>
<td>43500</td>
<td>23000</td>
<td>34,58</td>
</tr>
<tr>
<td>0,1</td>
<td>55000</td>
<td>30500</td>
<td>35,67</td>
</tr>
<tr>
<td>0,2</td>
<td>58500</td>
<td>42500</td>
<td>42,07</td>
</tr>
<tr>
<td>0,4</td>
<td>54500</td>
<td>38500</td>
<td>41,39</td>
</tr>
<tr>
<td>0,8</td>
<td>42000</td>
<td>40000</td>
<td>48,78</td>
</tr>
<tr>
<td>1</td>
<td>16000</td>
<td>30000</td>
<td>65,21</td>
</tr>
<tr>
<td>2</td>
<td>9500</td>
<td>41500</td>
<td>81,37</td>
</tr>
<tr>
<td>Control</td>
<td>70000</td>
<td>20000</td>
<td>22,22</td>
</tr>
</tbody>
</table>

### Table 2. Number and density of dead cells in the original solution of post-exposure to cisplatin 48 hours

<table>
<thead>
<tr>
<th>Concentration (mg/ml)</th>
<th>Observation 24-hour</th>
<th>Observation 48-hour</th>
<th>Observation 72-hour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Live</td>
<td>Dead</td>
<td>Death cell density (%)</td>
</tr>
<tr>
<td>0.05</td>
<td>92000</td>
<td>8500</td>
<td>8,45</td>
</tr>
<tr>
<td>0,1</td>
<td>85000</td>
<td>14500</td>
<td>14,5</td>
</tr>
<tr>
<td>0,2</td>
<td>73000</td>
<td>14000</td>
<td>16,09</td>
</tr>
<tr>
<td>0,4</td>
<td>91500</td>
<td>13000</td>
<td>12,44</td>
</tr>
<tr>
<td>0,8</td>
<td>44500</td>
<td>12500</td>
<td>21,92</td>
</tr>
<tr>
<td>1</td>
<td>35000</td>
<td>16500</td>
<td>32,03</td>
</tr>
<tr>
<td>2</td>
<td>34500</td>
<td>18500</td>
<td>34,90</td>
</tr>
<tr>
<td>Control</td>
<td>117500</td>
<td>3500</td>
<td>2,89</td>
</tr>
</tbody>
</table>
Discussion

In a population of tissues or cells, apoptosis and necrosis are two extremes of death cell. Low cisplatin concentrations that correlated with apoptotic death cell and high doses cause the death cell due to necrosis. Apoptosis is a response to cellular stress at the intensity of exposure below the necrotic threshold. High doses of cisplatin resulted in the damage of a number molecules that involved in the supply of cell energy adenosine triphosphate (ATP) 9.

The proteins directly involved or indirectly in the apoptotic process leading to the death of necrotic cells, as evidenced by the appearance of necrotic cell features at exposure to high doses of cisplatin resistant keratinocyte tissue. Exposure to high doses of cisplatin causes the reduction of ATP cell levels resulting in severe ATP depletion. Then, it will cause a rapid metabolic collapse resulting in necrotic death cell. The fewer ATP depletions correlated with lower doses of cisplatin cause apoptosis by the release of mitochondrial cytochrome 10.

Some studies suggest two lag phase in cisplatin cell growth inhibition in accordance with the results of this study that within the first 6 hours, no cisplatin inhibition effect was detected. It was estimated that in that period cisplatin accumulates and reaches the DNA genome to then express its pharmacological activity. After that period there was a rapid decrease in cell viability up to 20 post-exposure hours 11. The second lag phase of static cell/plateau growth occurs at 20-24 hours, which estimated to occur due to inactivation of cisplatin by thiol compound, only by then, there will be a significant decrease in cell viability. At the length of exposure duration up to 48 hours, there was an extensive membrane blockade of platelet function, the proportion of dead cells did not parallel with the drug content of assumption that saturation at the receptor has been achieved 12.

In the 48-hour duration of cisplatin exposure, cell proliferation has lasted for 2-4 generations of cells (assuming doubling the time of NPC cell line time in varies from 10.5 to 28.5 hours). The highest level of cisplatin uptake (passive diffusion) at the early time, that the more death cell at the beginning of the duration exposure with the proliferation of cells have lasted 2 to 4 cells generations within 48 hours, the number of dead cells becomes less than the new living cells resulting from the proliferation that occurs after the cell undergoes recovery 13.

The EC₅₀ indicator was the concentration or dose that required by a drug to achieve the desired effect of 50% in vitro. EC₅₀ for cytotoxicity means at concentrations of 50% cells showing the effect of death cell 14. Measurement of drug concentrations or doses usually follows a rapidly increasing pattern of sigmoid curves in relatively small dose changes. The effective dose point mathematically determined by drawing the corresponding line that was more easily determined by a graph than a complex statistical equation 15.

A number of studies used both EC₅₀ and IC₅₀ indicators with similar results. Another study reported that the cisplatin dose of 0.5 μg in the cell line of NPC CNE1 cellular was damage but the cells still respond actively that characterized by the inhibition of cell growth in the early period of observation but then recovered 16. Kadashiet conducted a study of cisplatin cytotoxicity on several cell culture types using IC₅₀ indicators obtained different values. Different doses that effect cytotoxic effects dissimilar between cell types because the mechanisms of apoptosis induced by cisplatin were unlike, and highly specific in each cell. This difference might also be due to the doubling time difference between cell types, especially in the growth-regulated neoplastic cells 17.

Conclusion

There was the correlation between the dose increased of cisplatin and cytotoxicity in NPC stem cells. Moreover, there was a correlation between the duration of cisplatin exposure and cytotoxicity in NPC stem cells. The effective dose of cisplatin resulting in a cytotoxic effect on NPC stem cells was by 1 μg at 24-hours exposure duration.

Ethical Clearance: This research involves participants in the process using a questionnaire that was accordant with the ethical research principle based on the regulation of research ethic regulation. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, non-maleficence, and justice.

Conflict of Interest: The authors have not found any conflict of interest related to this research so far.
Source of Funding

All of the cost and fees related with this research are paid by the authors only with no sponsorship nor external funds.

References


Optimization of Streptokinase Mutant Protein Purification Method Using Affinity Chromatography Technique

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Abstract

Protein purification has always been one of the most critical and challenging stages of drug-protein production. Streptokinase as the most common, and currently, the most cost-effective fibrinolytic drug is no exception. In this study, the mutated streptokinase producing clone (SK263cyc) to which the histidine tag was added was grown in TY2x medium, and SDS-PAGE assessed protein expression after induction of protein expression. Three different methods did protein purification; in the first one, metal, ion affinity chromatography (IMAC) technique was used. In the second solution, first, by filtration with ammonium sulfate, the purification was carried out, and then by affinity purification, chromatography continued. In the third solution, hydrophobic chromatography was used to purify the streptokinase protein. The purity of ophthalmic purity was 93.2%, and the purity of hydrophobic purity was about 90.4%, whereas the combination of pre-treatment with ammonium sulfate and the purity of the ophthalmic method did not achieve more than 88%. In general, the results of this study show that the IMAC method is more suitable as a final method in the process of streptokinase purification than the other two approaches.

Keywords: Streptokinase, Recombinant protein, Affinity chromatography, Hydrophobic gel purification, Ammonium sulphate

Introduction

When a blood clot forms in the circulatory system, it can cause blood clots, which can cause dangerous complications and, ultimately, death. A healthy balance system prevents clots from forming in a natural circulation system. The mechanism of blood clot formation is one of the most important and complex physiological systems in the body, and streptokinase is the most common and currently the most cost-effective fibrinolytic drug for this purpose. The most important part of designing a suitable “downstream process” for purification of a protein for analysis and treatment purposes is the separation of the desired protein from other available impurities and contaminants using various purification methods. The highest increase in concentration is due to the separation stage of the product, but the quality of the products dramatically increases during the initial purification stage. SK263cys is a mutant protein that contains cysteine amino acid at position 263 instead of glutamic acid and is designed to perform specific pegylation on cysteine amino acid. High purity protein is required for this process. Generally, in industrial processes and most research, the HIC method is used to purify streptokinase. Because this process is costly and low yield. In this study, purification of streptokinase protein was performed by applying and optimizing the IMAC method and combining it with one step pre-purification by ammonium sulfate deposition method. We also tried to purify this protein with the highest purity and higher efficiency.

Materials and Method

Protein Expression

The most commonly used host for the expression of Escherichia coli, the strain of Escherichia coli BL21 Rosetta (DE3), was used to express the mutant streptokinase protein. First, in a 5cc TY2x medium containing kanamycin antibiotic 50 ml / μg, a unique culture was prepared overnight. After 24 hours, after transferring the vertex tube, 100 μl of this medium was
transferred to 50 ml of fresh and warm TY2x medium containing kanamycin antibiotic and stirred at 150 rpm at 37 °C until ambient light absorption at 600 nm (600 OD) (0.4 to 0.6).

To produce protein and induce protein expression in large quantities, 50 µl of stock (M1 IPTG) was added to the culture medium to increase its concentration in 1 mM medium. The medium environment-friendly was returned to the incubator again and incubated for 3–4 h at 150 rpm at 37 °C. After incubation, the OD600 medium was re-recorded.

**Protein Purification**

Cell failure was performed by cell lysis (8 mM urea + 10 mM tris + 100 mM sodium dihydrogen phosphate). To prepare cell extracts from 50 ml TY2x culture medium, the culture medium was pre-divided into two falcons and centrifuged at 5000 rpm for 10 min.

The supernatant was discarded, and the precipitate was kept. To the precipitate was added 5 ml of lubricating buffer. After one to two hours, the crystalline contents of the Falcon were centrifuged at 5000 rpm for 10 minutes, and the supernatant was kept in the freezer after separation. The cell extract obtained was used in three strategies.

1: Hydrophobic Interaction Chromatography

2: Immobilized Metal Affinity Chromatography

The first strategy is the use of affinity chromatography without pre-purification, and the second strategy is the use of ammonium sulfate precipitation as the pre-purification and the affinity chromatography as the final purification. Both of these solutions were compared with the primary approach used in the industry.

Purification by Hydrophobic Interaction Method (HIC)

Gel filtration chromatography was performed using Sephadex-25G gel. By passing column equilibration buffer (20 mM tris, 0.2 mM sodium chloride and 8.5 = pH), the sample was prepared for injection, and then 5 mL of sample was slowly loaded into the column by the sampler.

Purification by Affinity Chromatography

One of the important features of this Purification system is its high capacity for binding to His-tag proteins (5-10 ml / mg) and insensitivity of the system to the precise 3D structure of the protein. For small-scale denaturing purification, after breaking the cell wall and extracting the cell contents, we centrifuge the liquid and load the supernatant after centrifugation on the chromatography column. After complete removal of buffer (pH 8), urea 8 mM + 10 mM tris + 100 mM sodium dihydrogen phosphate (5 ml sample was injected into the column by the sampler). When the sample was completely removed from the column, the washing buffer passed through the column. In the process of this study, pH of the buffer of the washing step was optimized in the range of 6.3 to 5.3 and a suitable pH of 5.7 was obtained. Figure 2b shows the effect of pH regulation on the efficiency of the IMAC method. At this point, proteins that are weakly bonded with nickel or have no bond at all with histidine are removed from the column. If the pH of the buffer used in the washout phase is not adequate, the target protein may also be removed from the column, resulting in a negative effect on the final purification. In the wash step, the buffer is introduced into the column at pH 4.5 to remove the target protein. The column outlet was collected in sterilized microtubes in a volume of 1 ml [4, 10].

2.2.3 Pre-Purification by Ammonium Sulfate Deposition and Purification by Affinity Chromatography: After preparation of the cell extract, the product was prepared with a pre-purification step by ammonium sulfate precipitation to enter the IMAC column. The amount of protein in the samples from each step of the process was determined by electrophoresis. Figure 5 illustrates these results. Table 3 also relates to the results of the gel analysis by software.

**Protein measurement**

**Method: SDS-PAGE**

To confirm the presence of the protein and to evaluate its value, the samples obtained from each step of the studied strategies were used by PAGE-SDS electrophoresis [7].

**Optical Method for Calculating Total Protein:**

OD of each sample was measured using a spectrophotometer at 280 nm. The mean ODs were obtained and multiplied by the dilution and extinction coefficient of 0.925 for streptokinase, and the total protein content of the sample was obtained [9].
Calculate the process efficiency for the target protein and its purity

The gel electrophoresis image of each process was analyzed by Lab Image software, and the percentage of protein purity was determined at each step; the purification efficiency is then calculated using the degree of purity.

degree of purity = Total Streptokinase / Total Protein Concentration * 100

Yield = Purified Protein Concentration * Degree of purity / total protein concentration

Results

The results of electrophoresis showed that the protein was produced properly (Fig. 2). In the HIC purification strategy, the urea in the protein solution was first removed (Fig. 1A), and then the isolated proteins were isolated from streptokinase by HIC using S-650 butyl gel (Fig. 1B).

In vitro analysis of purified samples showed that all three purification strategies performed well. After optimizing the pH of the wash buffer, the amount of protein produced by IMAC at a concentration of 1.71 mg / ml was higher than the other two methods (Fig. 2B). After careful examination using Lab Image software and determination of purity, it was found that the highest purity was related to IMAC 93% method. The results of electrophoresis showed that the protein was produced at the appropriate level at the beginning of the work.
Measurement of protein concentration by the HIC method showed that the highest amount of protein and the highest yield was obtained by the IMAC method, while the lowest yield was obtained by combined IMAC method and deposition with ammonium sulfate (Fig. 3A and B).

![Graph showing protein concentration and yield](image)

**Conclusion**

The results of this study showed that the IMAC method had better efficiency than HIC in purification of streptokinase with histidine tag. Also during the IMAC process based on the results, it was found that the pH of the wash buffer for the purification of streptokinase protein was 5.7.

**Ethical Clearance:** This project didn’t need any ethical permission.

**Source of Funding:** The project was co-funded through Islamic Azad University Pharmaceutical Sciences Branch, Tehran, Iran and Iranian-Australian Community of Science, Tehran, Iran.

**Conflict of Interest:** There is no conflict of interests in this project.

**Reference:**


Effect of Dietary Energy Density on Increasing Blood Glucose Pattern and Hunger-Satiety Sensation

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Abstract

\textbf{Background:} There was no data how these food density could effect on glucose and visual analog levels based on hunger-filled scales in Indonesian population.

\textbf{Objective} To determine the effect of food energy density on glucose levels and hunger-satiety sensation.

Material and Methods: Seventeen women 18-22 years of age with BMI> 25, were given low energy density breakfast (n = 9) and high energy density (n = 7). The subjects were fasted for 10 hours, fasting blood glucose levels was measured as well as hunger-satiety sensation with a visual analog scale (VAS), prior to treatment. Breakfast was started at 08.00 a.m chew up to 32 times for 15 minutes. Blood glucose levels and VAS measurement were taken back in 2 hours and 4 hours afterward.

\textbf{Result:} There was a significant differences in glucose levels between 4 hours postpandrial and 2 hours postpandrial (p = 0.031) and also between 4 hours postpandrial and fasting state (p = 0.042) in both groups. Analysis of hunger level VAS (p = 0.02) and satiety level VAS (0.04) at 4 hours postpandrial also differ significantly. Correlation analysis between hunger level VAS and blood glucose was different significantly (p = 0.01) with r = -0.59.

\textbf{Conclusions:} The low energy density foods produce a pattern of increasing blood glucose levels were more stable, thus, more able to suppress the sensation of hunger than high energy density.

\textbf{Keywords:} Energy Density, Blood Glucose Level, VAS, Hunger-Filled Sensation

\section*{Background}

Obesity and overweight increased very sharply, almost 35\% of the world’s adult population has been in this condition. Women are more susceptibility into the condition than in men. The obese population is beginning to stabilize in the United States, and its prevalence continues to increase in some countries in Europe and Asia. Obesity is a core component of the metabolic syndrome and includes the top ten health risk factors by WHO that are associated with chronic disease \textsuperscript{1}.

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The causes of obesity are multifactorial, including genetic, environmental, physiological, cultural, political and socioeconomic factors. Pathophysiological, obesity occurs when the balance of energy shifts in a positive direction and closely related to high calorie intake. The phenomenon of food that has high energy density also triggers a large calorice intake. Therapy used in limiting caloric intake during this time using sympathomimetic drugs that increase satiety or inhibition of fat absorption so that the calorice intake will decrease. But on the drug has side effects and can not be used in all conditions of the patient \textsuperscript{2}.

Indonesia has a high of food diversity. For example, the composition of Madura rice, an ethnic food in East Indonesia, has high fiber and low energy density. However, there was no data how these food density could effect on glucose and visual analog levels based on
hunger-filled scales. The aim of this study was analyzing the effect of energy density of food on blood glucose levels and Visual Analog Scale (VAS). Therefore, it can be used for prevention and therapy in handling obesity through the influence intake of calories into the body.

**Material and Method**

The study was conducted with a time series true experimental design under the approval of the Research Ethics Commission of Health Faculty of Universitas Airlangga (No. 284-KEPK). Subjects of the study were 17 people who participated and signed informed consent, BMI >25, women aged 18 to 22 years were given a low energy density breakfast (n = 9) and a high energy density diet (n = 7). Ten hours before breakfast, subjects were not allowed to eat, and measurements of fasting blood glucose and visual analog scale (VAS) were performed before breakfast. Breakfast was done at 08.00 then chewed up to 32 times for 15 minutes. Blood glucose and VAS levels were measured after 2 hours and 4 hours postpandrial.

**Food Energy Density**

Low food energy density is a low-calorie breakfast with a density of <1.6 calories/gram given food in the form of Madura corn rice that consisting of corn rice, pepes fish, fried fish, orem tempe, eggplant sauce, kothok salted fish, vegetable urap and water 200 ml. Madura maize rice used has a mature weight of 320 grams, with food density of 1.4 cal/g, protein 22.18 g (19.47%), fat 16.42 g (32.54%), carbohydrate 56.38g (49.51%), fiber 6.4 g with total energy 455.47 kal.

High energy density food is a low-calorie breakfast with a density >2.1 calories/gram given fast food meals consisting of fries, fried chicken flour, tomato sauce and 200 ml water. This food has a density of 2.2 cal/g, with mature weight of 200 g, protein 13.73 g (12.15%), fat 30.29 g (27.64%), carbohydrate 31.22g (27.64%), fiber 1.8 g with a total energy of 451.81 cal. Data were analyzed for distribution normality by Kolmogorov Smirnov test, mean difference test using independent t-test for normal distribution, and Mann Whitney test for abnormal distributed. Data analysis using SPSS version 16.

**Results**

Seventeen women were enrolled in this study with 18 to 22 year age range, with the distribution that are shown in table 1. Based on IMT, 55.55% of the low energy density group were categorized in BMI 25.0-29.9 whereas 62.5% of high energy density group were categorized in BMI of 30.0-34.9 (table 2).

From figure 2 and 3, it showed that fasting blood glucose levels in low energy density groups are higher than in high energy density groups. The low energy density group obtained a mean of fasting blood glucose by 65.67 ± 13.33 g/dL, 2 hours postpandrial by 81.89 ± 11.11 g/dL, and 4 hours postpandrial by 106.00 ± 56.00 g/dL. Meanwhile, density high energy has mean of fasting blood glucose of 72.00 ± 13.00 g/dL, 2 hours postpandrial of 83.38 ± 10.16 g/dL, and 4 hours postpandrial 96.75 ± 14.38 g/dL.

Figure 1 and 2 showed that low energy density group has higher glucose levels than high energy density group. Even in D2 and DT, there were significant differences between low and high energy density p <0.05, i.e., differences blood glucose levels of postpandrial between 4 hours and 2 hours D2 p = 0.031 and DT p = 0.042.

Visual Analog Scale is used to assess how hungry, satiety or appetite certain foods in a person. In table 3 we can see how the VAS of each question on fasting conditions, 2 hours postpandrial and 4 hours postpandrial. On examination of normality of data distribution got p >0.005 for all data except at third question at 4 hours postpandrial got p = 0.037. To see the average difference was done independent t test got significant difference on VAS number one at 4 hours postpandrial with value p = 0.02. A significant difference was also found in the mean VAS number three 4 hours postpandrial using the Mann Whitney test, value p = 0.037.

On the answer to question VAS number one is to assess how hungry the research subjects and the third question how much satisfied is obtained significant difference is done the correlation test on glucose levels in 4 hours postpandrial. The results can be seen in table 4. Additionally, from table 4 are shown that those who have hungry conditions (VAS1) correlated with blood glucose levels with p = 0.01. The lower the glucose level will be greater (R = -0.59).
Discussion

This study shown that breakfast with low energy density increases low blood glucose levels than high energy density levels at 2 hours postprandial, therefore this glucose levels tends to be maintained even slightly increased at 4 hours postprandial. There were a significant differences in D2 and DT where the mean of the differences in the low energy density group have higher glucose levels than high energy density group. This suggests that there was a stable increase in glucose levels of the low energy density group rather than high energy density. This result was similar to previous research that high-glycemic index diet will decline rapidly in glucose.

Decreased glucose levels in the high energy density group due to increased levels of hyperinsulinemia response by a rapid increase of glucose levels, thus, the decreased in glucose levels was faster than the low energy density group. This activity is known as glucostatic. The differences of corn fiber with fenugreek fiber were able...
to make a significant difference of glucose levels. The research by David stated that these fibers did not affect glucose levels postprandial compared to the control group 10.

The results of this study implied that low-energy food density have more stable and favourable pattern of glucose levels in short and medium-term of energy homeostasis regulation. The energy density of the VAS affects the sensation of hunger and satiety at 4 hours postprandial 11. The energy density does not affect the desire for certain types of food, such as sweet, salty or savory. If this condition was connected to the blood glucose levels, then food density will affect hunger sensation. Low energy density can maintain glucose levels in the blood better to suppress hunger sensation 12.

This result was similar with some previous studies, Sholehah (2019) suggested that high index glycemic carbohydrates make satiety sensation in a short period (within one hour), however low glycemic index levels will keep it moderate at about two or three hours after eating. Meanwhile, it was different from a study by Mathern that stating the provision of fenugreek fiber on the diet will lower calorie intake, increase satiety, and reduce hunger. Even though in both these satiety and hungry sensations have no correlation with postprandial glucose 5.

Hunger and satiety were affected by the glucose levels that circulating in the peripheral and cerebral and the volume strain by the fibers in gastrointestinal tract, although glucose levels have a greater role than fiber strain. It said that the method of giving glucose either orally or intravenously did not affect the sensation of hunger satiety measured by VAS. It seems that satiety is not by the way inlet of glucose into the body, but by blood glucose levels 13.

When an individu has mild hypoglycemia, the limbic-striatal region of the brain will be activated and will create a strong appetite for high-calorie foods. Meanwhile euglikemia was more activate in medial prefrontal cortex that could lead to reduce food stimuli, in the other hands, high levels of glucose will bring out more powerful stimulation 14. Thus, circulating glucose modulates stimulation regulation or barriers to food motivation. Increased of circulating glucose levels in the brain also increase functional connectivity between the hypothalamus, thalamus and striatum as well as decrease the cerebral blood flow thus full sensation will be achieved 15. The result of this was the low energy density food produces a more stable blood glucose levels that lead to decreased hunger sensation than in high energy density food 16.

Low energy density food have a higher fiber composition than high energy density food. Higher fiber in diet could increase the satiety within 1.5 to 2 hours after eating. Increased of satiety sensation is caused by the stimulation of satiety hormone production; peptide YY and glucoagon-like peptide-1. According to Oide et al. (2019), provision of high fermented fiber has a tendency to decrease the appetite. The provision of this fiber did not affect glucose levels, PYY, GLP-1 and ghrelin 17.

Based on the previous studies, the provision of a high glycemic index will increase hunger and reduce satiety in short-term interventions. However, this effect is varied when intervention performed in a long period. Increased blood glucose levels will trigger hyperinsulinemia and suppress ghrelin secretion. This study showed how the role of low and high energy density as an exposure to glucose levels, and hunger-satiety sensations. It has been found that low energy density food could maintain blood glucose levels and used as a way of decreasing caloric intake into the body. Further studies and explanations are needed if the low density food are exposed in the repeated frequency in a long term, thus its role in homeostasis energy will clearer.

**Conclusions**

The low energy density foods produce a pattern of increasing blood glucose levels were more stable, thus, more able to suppress the sensation of hunger than high energy density.

**Ethical Clearance:** This research involves participants in the process using a questionnaire that was accordant with the ethical research principle based on the regulation of research ethic regulation. The present study was carried out in accordance with the research principles. This study implemented the basic principle ethics of respect, beneficence, non-maleficence, and justice.

**Conflict of Interest:** No conflict or competing interest related with this article until now.
Source of Funding: We pay all of the sources of our own research without sponsorship from other.

References

Effect of Phenytoin Usage Duration Against Hs-Crp Levels in Epilepsy Patients

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Abstract

Background: Patients with epilepsy have a higher risk of death than the normal population. Epilepsy patients have increased mortality due to cardiovascular disease with standardized mortality ratios ranged between 1.2 and 2.5. The incidence of non-fatal coronary heart disease also increased significantly between 34% and 63%. Atherosclerosis as an inflammatory state has an important biomarker namely hs-CRP. Long-term use of phenytoin will have an effect on hs-CRP. hs-CRP is an atherosclerotic biomarker with a better cardiovascular predictor than blood lipid and homocysteine levels

Methods: This study was conducted in epilepsy patients who fulfill ESR the criteria of inclusion and exclusion in Outpatient Unit of Dr. Soetomo General Hospital from October 2014 to April 2015. Patients were divided into a control group and case group. The control group was patients with hs-CRP levels <1.7 meanwhile, control group was >1.7. Tracking of phenytoin usage duration was performed in both groups.

Results: Thirty-four subjects were enrolled in this study which consisted of 21 males (61.76%) and 13 females (38.23%). The mean age of subjects in the case group was 31.6 ± 12.6 years and control group was 26.5 ± 11.3. Data of phenytoin usage duration and hs-CRP levels were analyzed.

Conclusion: There was no significant difference between hs-CRP levels on phenytoin usage duration of >2 years and <2 years with p = 0.290

Keywords: Phenytoin Usage, Hs-Crp Levels, Epilepsy Patients

Introduction

Patients with epilepsy have a higher risk of death than the normal population. It was found a positive correlation between epilepsy and vascular comorbidity with mortality of 5.3 and morbidity up to 71. This was a reason why this group has a higher risk of death. Epilepsy patients have increased mortality due to cardiovascular disease with standardized mortality ratios (SMRs) between 1.2 and 2.5. The incidence of non-fatal coronary heart disease also increased significantly between 34% and 63%. An increased mortality caused by cardiovascular disease in epilepsy patients They found an increased mortality in patients with epilepsy who used the old type of anti-epilepsy drugs. Studies conducted on epilepsy group treated with phenytoin found intima media thickening of the carotid artery. This was a risk factor for the incidence of cerebrovascular and cardiovascular diseases. This supports the study that atherosclerotic factors were involved in vascular death and morbidity in epilepsy patients.

It was needed to measure the thickening of the media tunica and intima tunica in the carotid artery in order to determine the presence of atherosclerosis. This measurement in epilepsy patients treated with phenytoin showed a strong association with the risk of stroke and myocardial infarction at all ages. There was a significant increase in the thickness of the media tunica and intima tunica in epilepsy patients with phenytoin therapy. Long-term use of phenytoin has altered metabolic...
function thus it increases atherosclerosis in epilepsy patients. He further mentioned that long-term use of phenytoin has been found to increase the thickness of the intima tunica and the media tunica of the carotid artery. Atherosclerosis as an inflammatory state has an important biomarker namely hs-CRP. hs-CRP increased in patients with epilepsy phenytoin. There was a conducted study about changes in the use of anti-epilepsy drugs (AEDs). They substituted carbamazepine or phenytoin with non-enzyme-inducing AEDs then they found that hs-CRP levels dropped to > 30%. This results suggested a substantial role of phenytoin to the atherosclerosis process. This process underlies the occurrence of cardiovascular and cerebrovascular disorders thus it is necessary to examine the biomarkers of atherosclerosis in epilepsy patients with phenytoin usage. Atherosclerotic biomarker was better as cardiovascular predictors than blood lipid levels and homocysteine. Professionals from the National Academy of Clinical Biochemistry mentioned that hs-CRP was the most acceptable biomarker and also served as a marker. There has been atherosclerosis that may progress to cardiovascular and cerebrovascular disease. The correlation between phenytoin usage duration and the levels of hs-CRP in the blood remains unclear. Phenytoin has an influence on the immune system by stimulating macrophages to secrete IL-1 and IL-6. IL-1 and IL-6 will stimulate hepatocyte cells to secrete hs-CRP. Therefore, the presence of hs-CRP is a result of phenytoin and the process of atherosclerosis.

Method

This study was an observational analytic using a case-control study design that was performed from June 2014 to April 2015 in the Neurology Outpatient Unit of Dr. Soetomo General Hospital Surabaya. The subjects were all epilepsy patients who received Phenytoin therapy and fulfill ESR the inclusion and exclusion criteria. The sampling technique used was consecutive sampling.

Preliminary data were obtained by performing anamnesis, physical and neurological examination, and confirmation of medical records. All recorded data were collected for data tabulation and statistical analysis. Collected categorical data were analyzed using chi-square test with α = 0.05. Meanwhile, the normality test was performed for numerical with Saphiro Wilk test. Numerical data with normal data distribution (age, BMI, leukocytes, GD2) was analyzed using an unpaired t-test, whereas, numerical data with abnormal data distribution was analyzed using Mann Whitney U test in order to determine differences in both groups.

Results

Subjects Characteristics by Sex

There are two characteristics of subjects, such as male and female. Thirty-four subjects were enroll ESR in this study that consisted of 21 males (61.76%) and 13 females (38.23%). The case group has male subjects by 10 people (58.8%), it was less than in the control group by 11 people (64.7%), meanwhile female subjects in case group was 7 people (47.6%), more than control group that has 6 people (35.3%) (p = 0.724). Different percentage of sex in each group was not statistically significant (p = 0.724)

Subjects Characteristics based on Leucosyt Amount

Table 1. Subjects Characteristics based on Leucosyt Amount

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<th>Group</th>
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<tbody>
<tr>
<td></td>
<td>Case</td>
<td>Control</td>
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<tr>
<td>Mean</td>
<td>6.78</td>
<td>7.222</td>
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<tr>
<td>SD</td>
<td>1.97</td>
<td>1.53</td>
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<td>P</td>
<td>0.460</td>
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The number of leukocytes in the case group was 6.781 ± 1.970 years, it was higher than the control group by 7.229 ± 1.53 years. It was found that the data distribution of leukocytes was normal (p = 0.428) in both case and control groups. 2-independent sample T-test was conducted to identify the difference of leukocyte in case and control group and it was found that there was no significant difference with p = 0.460.
Subjects Characteristics Based on Postprandial Blood Sugar (GD 2)

Blood sugar levels 2 hours post-prandial in case group was $96.529 \pm 16.144$, it was slightly higher than control group by $84.564 \pm 12.631$. It was found that the data distribution for blood sugar 2 hours post-prandial was normal with $p = 0.149$ for case group and $p = 0.253$ for the control group. Therefore, 2-independent sample T-test was conducted to identify the difference of blood glucose in case and control group, thus it was found that there was no significant difference between two groups with $p = 0.279$ (Table 2).

Table 2. Subjects Characteristics based on Postprandial Blood Sugar (GD 2)

<table>
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<tr>
<td>SD</td>
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<td>Blood Sugar</td>
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The distribution of data for fasting blood sugar was abnormal ($p = 0.011$ for case group and $p = 0.627$ for the control group). The mean difference test to identify using Mann Whitney U test found that there was no significant difference of fasting blood glucose in case and control groups with $p = 0.634$ (Table 3).

Table 3. Subjects Characteristics based on Fasting Blood Sugar (GD 1)

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<tr>
<td>SD</td>
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<td>6.02</td>
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<td>Fasting Blood Sugar</td>
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Subjects Characteristics Based on Erythrocyte Sedimentation Rate (ESR)

The ESR in the case group was $8.41 \pm 4.89$; it was higher than the control group by $3.76 \pm 2.704$. It was found that the data distribution for ESR was not normal ($p = 0.150$ for case group and $p = 0.02$ for the control group). Mann Whitney U test was performed to identify the difference between LED in case and control groups. There was a significant difference with $p = 0.003$ (Table 4).

Table 4. Subjects Characteristics Based on Erythrocyte Sedimentation Rate (ESR)

<table>
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</table>

Subjects Characteristics Based on Phenytoin Usage Duration

Based on the duration of phenytoin usage, 12 (70.6%) subjects with phenytoin therapy >2 years were found in the case group; it was more than the control group by 9 subjects (52.9%). Subjects with phenytoin therapy <2 years from the case group was 5 subjects (29.4%); it was less than the control group by 8 subjects (47.1%). The differences in case and control groups were not statistically significant with $p = 0.364$ (Table 5).
Tabel 5. Subjects Characteristics based on Phenytoin Usage Duration

<table>
<thead>
<tr>
<th>Duration</th>
<th>Group</th>
<th>Total</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case</td>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>&gt;2 years</td>
<td>12 (70.6%)</td>
<td>9 (52.9%)</td>
<td>21</td>
</tr>
<tr>
<td>&lt;2 years</td>
<td>5 (29.4%)</td>
<td>8 (47.1%)</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>17 (100%)</td>
<td>17 (100%)</td>
<td>34</td>
</tr>
</tbody>
</table>

Effect of Phenytoin Duration >2 years and <2 years against hs-CRP Levels

There were 12 people (70.6%) who used phenytoin for >2 years in the case group, it was higher than in the control group by 9 people (52.9%). Meanwhile, there were 5 people (29.4%) in the case group who used phenytoin for <2 years, it was less than the control group by 8 people (47.1%). This difference was not statistically significant with p = 0.364 (Table 6).

**Table 6. Effect of Phenytoin Duration >2 years and <2 years against hs-CRP Levels**

<table>
<thead>
<tr>
<th>Duration</th>
<th>Group</th>
<th>P</th>
<th>OR (CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;2 years</td>
<td>12 (70.6%)</td>
<td>0.290</td>
<td>2.133 (0.519 – 8.71)</td>
</tr>
<tr>
<td>&lt;2 years</td>
<td>5 (29.4%)</td>
<td>8 (47.1%)</td>
<td>34</td>
</tr>
</tbody>
</table>

This suggested that the use of phenytoin for >2 years has no significant relationship to elevated levels of hs-CRP (95% CI, OR = 0.516-8.761). It indicated that the use of phenytoin for >2 years was 2.133 times more frequent to increase hs-CRP compared to the use of phenytoin for <2 years (Table 9).

**Discussion**

We found no significant differences in basic characteristics of subjects. Clinical exclusion data of subjects in the form of ESR, leukocyte, GDI/GD2 were analyzed to eliminate bias against hs-CRP. The subjects were 34 patients that consisted of 21 males (61.76%) and 13 females (38.23%). This result was in accordance with epidemiological data in Indonesia which stated epilepsy occurred more frequently in male than female. The mean age in the case group was 31.06 ± 11.3 years and the control group was 26.53 ± 11.3 years, there was no significant difference in both groups with p = 0.278. This demographic data were compared to a study by Teng-Yeow Tan, Cheng-Hsien Lu et al, which stated the mean age was 36.0 ± 11.3 in the case group and the control group was 36.2 ± 11.2 with p = 0.838. Age range was almost similar because older subjects often had accompanied metabolic disease which became one of the exclusion criteria.

Chi-square test on BMI, the number of leucocytes, GD1, GD2 did not obtain any significant difference between case and control groups. This indicated that
there was no effect of BMI, the number of leukocytes, GD1 and GD2 on hs-CRP levels in this study. In contrast, Teng-Yeow Tan et al. study did not control the BMI variable although Simionescu stated that fasting blood glucose, BMI, systolic blood pressure affected hs-CRP12. Subjects with increased ESR levels above the normal level have been excluded, however, higher ESR levels was obtained in case group that by 8.41 + 4.89 than the control group by 3.76 + 3.12. There was a significant difference (p = 0.003) of ESR levels in case and control group. It was mentioned that increased production of hs-CRP will increase the blood viscosity, thus it was associated with increased levels of ESR.

There were 12 subjects (70.6%) who used phenytoin >2 years in the case group, it was more than in the control group by 9 people (52.9%). Meanwhile, there was 5 subjects (29.4%) in the case group who used phenytoin <2 years, it was less than the control group by 8 subjects (47.1%). The difference was not statistically significant p = 0.290 thus it indicated that the use of phenytoin for >2 years had no significant correlation to the increase in hs-CRP levels.

The function of drugs in the body is influenced by the activity of CYP 450 in the liver. The different levels of CYP 450 affect the activity of drugs in the body. Phenytoin is an inactive metabolite. CYP 1A2A will hydrolyze phenytoin to active hydroxyphenitoin14. CYP 450 has various variants. The emergence of some types of the allele is due to the occurrence of several mutations that occur in the reproductive cells of each individual. The difference of alleles in the individual is caused by the changes in the structure of DNA bases such as the change of one of the DNA bases, the deletion or the rearrangement of DNA in one of its locus of chromosome15.

One variant of the CYP 450 is CYP 1A2A. CYP 1A2A is manufactured in the heart and is induced by some drugs such as oral contraceptives, coffee and cigarettes. That activity requires higher doses of phenytoin in smokers and female who use oral contraceptives. It was found that there were different types of phenotypes in each race. There was low CYP 1A2A activity <5% in Asian and African compared to CYP 1A2A activity in Caucasian. A Higher dose is required for phenytoin in Caucasian than in Asian16.

Cholesterol synthesis is influenced by CYP 51 A1 gene. A Higher dose of phenytoin leads to greater induction of the CYP 51 A1 gene, thus cholesterol production is also increased. This mechanism contributes to the onset of atherosclerosis.

**Conclusion**

There was no difference between hs-CRP levels in epilepsy patients who used phenytoin for >2 years and phenytoin for less than 2 years in Dr. Soetomo General Hospital Surabaya.

**Ethical Clearance:** This study protocol was approved by ethical clearance Dr.Soetomo Surabaya, Indonesia teaching hospital research.

**Conflict of Interest:** This study protocol was approved by ethical clearance Dr.Soetomo Surabaya, Indonesia teaching hospital research.

**Source of Funding:** This study is done with individual funding.

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3. Simionescu MAVS. Morphology of atherosclerotic lesions. *In Inflamation and atherosclerosis* 2012..


Comparison of Clinical Severity Improvement Degree between Acute Thrombotic Stroke Patients with Low and High Matrix Metalloproteinase-9 Levels

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Abstract

Background: Stroke is a cause of disability and dependency in both humanity and economic. Studies on the association of Matrix metallopeptidase 9 (MMP-9) levels with improved clinical severity are currently limited.

Objectives: To determine the improvement of clinical severity in acute thrombotic stroke patients with high and low MMP-9 levels.

Methods: Patients acute thrombotic stroke were grouped into low and high MMP-9 levels. MMP-9 levels were considered low when <840 ng/mL and high when ≥840 ng/mL. Clinical severity improvement was assessed using the ΔNIHSS scale.

Results: Eighty subjects were enrolled that consists of clinical severity improved patients with lower MMP-9 (37.9%) that higher than high MMP-9 (21.6%). This difference was not statistically significant (p = 0.115).

Conclusion: There was no difference in clinical severity improvement in patients with acute thrombotic stroke with low MMP-9 levels in blood serum compared to high MMP-9 levels.

Keywords: Acute thrombotic stroke, MMP-9, NIHSS Level

Introduction

Stroke is the main cause of death and disabilities in Indonesia and the world. Stroke has enormous clinical, social, and economic implications and demands a significant effort. The average mortality in 30 days for stroke is about 7.6%. There are currently four million people in the United States living with physical limitations due to stroke and about 15-30% of them suffer from the permanent disability. In 1990 the death rate caused by the stroke was 4.5 million worldwide.

Elderly with hypertension have a greater risk for ischemic stroke. The number of stroke patients will increase every year, it is estimated that the number of stroke patients will increase to 7.7 million by 2020. Todays, strokes not only affect the elderly population but also young adults and productive. Stroke could cause disability and dependency in both human and economic, impaired cognition and death function. Stroke survivors who could survive up to 30 days after the attack were about 20% and it requires further treatment. The cost of stroke treatment during acute phase treatment to long-term care in the United States is approximately US $ 51.2 billion every year. Several studies have demonstrated an inflammatory process that involved in the pathogenesis of stroke. Brain blood flow that stops could cause death in nerve cells that trigger an immune response that causes inflammation, cell activation, and infiltration.

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Once activated, inflammatory cells release various cytotoxic agents including the Metalloproteinase Matrix (MMP) which, if in the active state it could cause further cell damage and disturbances in the blood-brain barrier. Among some MMP, MMP-9 has a more dominant role in stroke.

MMP levels in circulating plasma could be used as a marker of stroke in predicting bleeding in embolic stroke, predicting clinical outcomes and the magnitude of infarct volume. The elevated MMP-9 levels after the occurrence of brain ischemia in the plasma were positively correlated with the severity of stroke as measured by the National Institutes of Health Stroke Scale (NIHSS) scores. However, until now there has been no study in Indonesia that observed the correlation between the magnitude of clinical severity improvement with low and high MMP-9 levels in patients with acute thrombotic stroke. Then, if this study proves the existence of such linkage, then the MMP-9 examination could be used as an alternative examination that helps explain the prognosis of healing stroke thrombotic acute in patients.

**Method**

An observational analytic study using case-control design was used, which was implemented in January to August 2015 in Seruni room, in Emergency Unit, Seruni A, Seruni B of Dr. Soetomo General Hospital Surabaya. The samples used in this study were those with acute thrombotic stroke as well as meeting the inclusion and exclusion criteria. Sampling technique used was consecutive sampling.

The inclusion criteria were: Patients with acute thrombotic stroke with onset of attack between 24 to 72 hours, age over 18 years, willing to participated the research (signed informed consent). Patients with acute thrombotic stroke who have sepsis, liver disorder or renal failure were exclude from study. The independent variables were MMP-9 in serum and the dependent variables were Clinical severity measured by NIHSS, while confounding variables were Smoking, hyperglycemia and hypertension.

This study used venous blood research material from patients with acute thrombotic stroke that needed to determine the MMP-9 level in serum. The research started by taking data of all subjects included in the inclusion and exclusion criteria and the responsible family of the patient (spouse, child, parent, close relative or another family part) briefed on the purpose, usefulness, and risk of the study, then the subject was requested to follow the research without coercion.

Preliminary data were obtained by taking anamnesis, physical examination and neurology, examination of MMP-9 level in *prodia* laboratory Surabaya, examination of clinical severity degree were assessed twice, at the same time with blood taking for MMP-9 (onset 24 to 72 hours) examination and at day 5 onset by using the NIHSS method. All recording results are collected for further data tabulation and statistical analysis then, the collected categorical data were analyzed by using odd ratio test. This research have been proposed of ethical clearance in the Research Ethics Committee of Dr. Soetomo General Hospital.

**Results**

**Basic data**

The means age in the group with the improvement of clinical severity was 51.23 ± 10.708 years and with no improvement was 57.02 ± 10.838 years. The mean age difference in both groups was not statistically significant with p = 0.35 (Table 1).

In the group with clinical severity correlation, the male subject was 10 (24.4%), it smaller than the female gender subject by 12 (30.8%). This difference was not statistically significant with p = 0.523 (Table 1).

Improvement of the severity in subjects group with normotension was 9 (64.3%), it bigger in a group of subjects with hypertension by 13 (19.7%). This was statistically differented with p = 0.001 (Table 1).

Improvement of clinical severity in group of subjects with normoglycemia was obtained by 14 (32.6%) subject, it higher than in the subjects group with hyperglycemia by 8 (21.6%). This difference was not statistically significant with p = 0.275 (Table 1).

The improvement of clinical severity in the non-smoking subjects group was 17 (29.8%), it higher than in the subjects who smoked as 5 (21.7%) was found. The differences in both groups were not statistically significant with p = 0.464 (Table 2).

The improvement of clinical severity in the subjects group with low MMP-9 levels was 11 (37.9%) subject,
it higher than in the subjects group with high MMP-9 levels by 11 (21.6%). The differences in both groups were not statistically significant with \( p = 0.115 \). It also obtained the odds ratio by 2.222 (CI 95% 0.814-6.064) with the \( \beta \) error was 38% (Table 3).

**Table 1. Basic Characteristics of Subject**

<table>
<thead>
<tr>
<th>A NIHSS</th>
<th>Improved n = 22</th>
<th>Constantly n = 58</th>
<th>Total n = 80</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years old)</td>
<td>51.23±10.708</td>
<td>57.02±10.838</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td>0.523</td>
</tr>
<tr>
<td>Male</td>
<td>10 (24.4.0%)</td>
<td>31 (75.6%)</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>12 (30.8%)</td>
<td>27 (69.2%)</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Blood pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normotension</td>
<td>9 (64.3%)</td>
<td>5 (35.7%)</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>13(19.7%)</td>
<td>53(80.3%)</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>Random Blood Sugar</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normoglycemia</td>
<td>14 (32.6%)</td>
<td>29 (67.4%)</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>8 (21.6%)</td>
<td>29 (78.4%)</td>
<td>37</td>
<td></td>
</tr>
</tbody>
</table>

| Smoking Status | | | |
| Un-smoking | | | 57 |
| Smoking | | | 23 |

**Table 2. Comparison of Smoking and Clinical Severity Improvement (ΔNIHSS)**

<table>
<thead>
<tr>
<th>ΔNIHSS</th>
<th>Total p</th>
<th>RO (CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improved n = 22</td>
<td>Constantly n = 58</td>
<td></td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Un-smoking</td>
<td>40 (70.2%)</td>
<td>57</td>
</tr>
<tr>
<td>Smoking</td>
<td>17 (29.8%)</td>
<td>5 (70.2%)</td>
</tr>
</tbody>
</table>

**Table 3. Comparison of MMP-9 Levels by Clinical Severity Improvement (ΔNIHSS)**

<table>
<thead>
<tr>
<th>MMP-9</th>
<th>ΔNIHSS</th>
<th>Total</th>
<th>P</th>
<th>RO (CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Improved n = 22</td>
<td>Constantly n = 58</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-MMP-9</td>
<td>11 (37.9%)</td>
<td>18 (62.1%)</td>
<td>29 (100%)</td>
<td>0.115</td>
</tr>
<tr>
<td>High-MMP-9</td>
<td>11 (21.6%)</td>
<td>40 (78.4%)</td>
<td>51 (100%)</td>
<td></td>
</tr>
</tbody>
</table>


Discussion

The mean age in the group with the improvement of clinical severity was 51.23 ± 10.708 years and in the group of no improvement was 57.02 ± 10.838 years. In accordance with literature that the highest incidence of ischemic stroke was at the age of 20-54 years and tended to decrease in the older age group. The incidence of stroke every 10,000 population increased from 22% (age group 45-55 years) to 32% (age group 55-64 years) and 83% (age group 65-74 years). The difference between each group was not significant (p = 0.35).

The sex ratio of the men subjects was 41, it higher than women by 39. It was in accordance with epidemiological studies that men have a higher risk of stroke than women, especially in the age group less than 65 years. Murphy et al also revealed that the incidence of stroke in men was 1.25 times higher than for women. Several studies have also reported that men were more likely to suffer a stroke caused by a disruption of large or small blood vessels. In this study, there was no difference in sex proportion between the two groups (p = 0.523).

Hypertension is a major risk factor for stroke and an increase in blood pressure often occurs in acute stroke. 50% of thrombotic stroke patients have hypertensive risk factors. The response of acute hypertension is an increase in blood pressure above normal and usually occurs within the first 24 hours in stroke patients. Increased blood pressure reaches 75-80% in acute stroke patients and will decrease spontaneously within a few days. Increased blood pressure in the acute phase of thrombotic stroke will provide severe output. Studies conducted on experimental animals with acute thrombotic stroke showed that autoregulation of cerebral blood flow in the penumbral area was impaired. Increased blood pressure is a natural response to increased perfusion and collateral blood flow in the penumbra to prevent ischemic damage.

Based on the analysis of clinical data obtained a significant result (p = 0.001). This was in accordance with research conducted by Kvistad CE et al. that there was a correlation between increased blood pressure and stroke severity when the patient was admitted to the hospital. A study conducted by Ishitsuka K et al., against 1874 patients with acute thrombotic stroke performed blood pressure measurement in 24 hours onset of stroke, it has resulted in a correlation between hypertension with poor outcome and hypertension with the minor improvement of clinical severity.

IST (International Stroke Trial) conducted by Leonardi et al. performed randomly in 17,398 patients with thrombotic stroke that showed a correlation between systolic blood pressure at the onset of acute stroke with death and disability in U-shaped (U-shape relationship) American Diabetes Association and Clinical Endocrinological Consensus The American correlation define hyperglycemia in patients undergoing hospitalization as hyperglycemia stress in which blood glucose levels> 140 mg/dL without the prior history of diabetes. Patients with hyperglycemia stress were often found in acute thrombotic stroke because in the acute phase there was the release of cortisol and norepinephrine which will manifest the decrease in insulin. Stress hyperglycemia might improve if the acute phase of a stroke was passed and 60% of hyperglycemia sufferers might become diabetic within a year.

When comparing between normoglycemia and the improvement of clinical severity in this study, the results were not significant (p = 0.275). Not much research data suggests that lowering blood sugar levels could improve the outcome. The largest study was a decrease in blood glucose levels with potassium-glucose-infusion infusion compared to standard saline infusion that showed no improvement in output and decreased mortality in patients.

Based on cohort studies conducted by Ueshima H et al, there were several things that could increase the risk of stroke and the occurrence of intracranial also extracranial atherosclerosis, including the number of cigarettes smoked in one-day, how long a person smokes (in years) and current smoking conditions. In this study, when comparing the non-smoking history and clinical severity improvement (ΔNIHSS), the results were not significant (0.464). This was in accordance with research conducted by Altafi D et al. that compared clinical outcomes (NIHSS) of thrombotic stroke smokers and non-smoking thrombotic stroke patients who resulted in a lack of correlation between thrombotic and non-smoking thrombotic stroke patients with clinical outcomes.
MMP-9 is a zinc-dependent proteolytic enzyme that significantly increases its cadmium in patients with thrombotic stroke (Castellanos M, et al., 2003). Based on a study conducted by Lisovaya OA it was found that elevated MMP-9 levels were not only correlated with an increase in cardiovascular incidence but were also associated with long-term vascular remodeling phenomena (Lisovaya OA, 2014).

The final result showed that the improvement of clinical severity in subjects with low MMP-9 level was 37.9% higher than in the subjects group with the high MMP-9 level by 21.6%. The differences in both groups were not statistically significant with \( p = 0.115 \) and the odds ratio value was 2.222 (CI 95% 0.814-6.064). This was not in accordance with the proposed research hypothesis. Inappropriateness with the proposed research hypothesis could be due to several things.

First, this study did not consider the infarct volume into the subject. Montaner J et al., Conducted a study of 39 thrombotic stroke patients evaluated at onset of stroke, 12, 24, and 48 hours of onset, and found that MMP-9 values had a positive correlation with NIHSS and infarct volume also revealed that MMP-9 was a strong predictor in predicting infarct volume \(^9\). Ning et al., Through a study conducted on 52 thrombotic stroke patients, explained that MMP-9 has a correlation with infarct volume and clinical severity \(^{21}\).

Second, in this study did not distinguish the location of the infarction that could affect the output of stroke in the study subjects. Montaner et al., Explains that there was a correlation between MMP-9 and the location of the infarct \(^9\), it was similar to a study conducted by Lucivero et al., In 29 thrombotic stroke patients, explained that high MMP-9 levels had a correlation with partial anterior cerebral infarct compared to cerebral infarct lacunar \(^{22}\).

Third, MMP-9 might be affected by the stiffness of arterial blood vessels. Arterial vascular stiffness could have an effect on hemodynamic disorders that trigger blood vessel remodeling and increase cardiovascular risk. Yasmin et al., Conducted a study of 116 patients with hypertension by measuring pulse wave velocity (PWV) in the aorta and brachial also measured MMP-9 levels and serum elastase activity (SEA) levels. From a study by Yasmin et al., Stated that arterial vascular stiffness was significantly correlated with MMP-9 levels (Yasmin et al., 2005).

**Conclusion**

There was no difference in clinical severity correlation in patients with acute thrombotic stroke with low MMP-9 levels in blood serum compared to the patients with acute thrombotic stroke with high MMP-9 levels in blood serum.

**Conflict of Interest**: There is no conflict of interest

**Source of Funding**: This study is self funded

**Ethical Clearance**: This study was approved by Ethical Commission of Health Research Faculty of Medicine University of Airlangga

**References**


The Effect of Model Intervention Towards the Intention of Early Detection of Cervical Cancer with Via Test

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Abstract

The purpose of this study was to evaluate the woman of childbearing age decision’s model towards the intention of early detection of cervical cancer with the VIA (visual inspection with acetic acid) test. The design was quasi-experimental. The population of women of childbearing age and sample size was 70. Technique: Paired samples statistics. Statistical analysis using the Wilcoxon test with a probability of 0.05. The difference in the value of the pre and post test were seen from the difference in the mean value between the pre and post test. In the treatment group, the difference test results were significant between the pre and post test, the value of p = 0.000. In Control group, the results of the difference test were not significant between the pre and post test, the value of p > 0.05. The Decision Model was a new finding of research where have the highest level of situation awareness on the situation, events and dynamics of its health condition. The decision model showed that with a high level of awareness, the women decided to make early detection of cervical cancer supported by high self efficacy and intention.

Keywords: Model, Intervention, Early Detection of Cervical Cancer

Introduction

Deaths from cervical cancer are projected to increase by almost 25% over the next 10 years. Based on the Globocan estimate, the 2012 International Agency for Research on Cancer (IARC), the incidence of cancer in Indonesia 134 per 100,000 people with the highest incidence in women was breast cancer by 40 per 100,000 followed by cervical cancer 17 per 100,000 and colorectal cancer 10 per 100,000 women(1).

WHO mentioned four important components that became pillars in the treatment of cervical cancer, namely: prevention of HPV infection, early detection through increased alertness and organized early detection programs, diagnosis and treatment, and palliative care for advanced cases. Early detection of cervical cancer includes an organized screening program targeting women of childbearing age groups, the establishment of an effective referral system at each level of health services, and education for health workers and women of productive age. Pre-cancerous lesion screening and treatment requires a lower cost compared to treatment and management of cervical cancer.

The target of early detection of cervical cancer was 50% of women until 2019, early detection programs in Indonesia were prioritized for women aged 30-50 years. The fact was that the coverage of the results of activities from 2007 to 2014, namely the achievement of early detection of cervical cancer screening was 2.45%(1).

In 2030 there will be a surge in cancer patients in Indonesia up to seven times(2). The number of cancer patients who died also became increasingly alarming. The number of cervical cancer cases in Indonesia is getting worse because more than 80% of cases that come to the hospital are at an advanced stage.

By the complex problem, the idea arose to screen cervical cancer with a simple method, namely VIA.

In dr. Pirngadi Medan Hospital, In 2007 there were 345 cases, in 2008 there were 25 cases, in 2009 were 48 cases and in 2010 were 40 cases. The high rate of cervical cancer patients in Indonesia was caused by this disease did not cause symptoms and low awareness of women to check their health(1). If more women were accustomed to doing early detection, and the disease has
spread to someone, it can be dealt with more quickly(3).

Behavior factors which include predisposing factors, supporting factors and driving factors. Other factors that affect the efforts of early detection of cervical cancer are individual factors that influence the woman to detect early cervical cancer, namely SA (situation awareness). SA was a continuous process that involves assessing events in the environment so as to give meaning to information to help decision. Comprehension of cervical cancer hazards and the purpose of screening, assisted in making decision about screening, was an important factor in whether respondents made choices regarding information about screening(5). Determinants influence intention on the behavior of early detection of cervical cancer was significant, one of which was perception of the national cancer screening program as well as perceptions about the risk of cervix cancer(6).

There was an effect of attitude on intention to do early detection of cervical cancer(7). The implementation of the VIA program mentioned based on the recapitulation of VIA examinations in Pematangsiantar, which showed the percentage of woman participating in the 2015 - 2016 VIA test and was not seen achieving the VIA test target for each puskesmas. By the national percentage that has attended the VIA test was still far below the effective screening coverage of 50%, even far below the target coverage of Pematangsiantar Health Department’s screening coverage of 20% per year.

Self-efficacy was an individual’s belief about his ability to organize and complete a task, mastering the situation needed to achieve certain results. Some dimensions of self-efficacy), namely magnitude, generality, and strength(8). The addition of self-efficacy increases the predictability of the TPB. Self-efficacy was the strongest predictor of intention(9).

This study, wanted to expand the research by integrating the framework of TPB. Ajzen (1991) with Theory Situation Awareness (Endsley, 1988), and Social Cognitive Theory explains that the TPB model was very open to add another predictor variable to predict intention and behavior about the object under study(10-11). This study will examine the decision model of women to perform early detection of cervical cancer with the VIA test.

Material and Method

The study design was quasi-experimental. The population of women of childbearing age and sample size was 70. Research variables were: perceptions, comprehension, attitude, subjective norms, projection, self-efficacy, intention and decision making intention to perform VIA within such specific period.

The data of the study consists of primary and secondary data. The primary data had obtained from interviews with spreading questionnaires. The obtained data through interview consists the data of projection, self-efficacy, intention, toward decision to perform VIA. Before, the instruments were used to measure each variable, then the necessary act would include performing an instrument test, which had performed validity test and questionnaire reliability.

Statistical analysis using the Wilcoxon test with a probability of 0.05. To analyze the difference in mean and standard deviation before and after treatment in each intervention and control group, a statistical analysis was performed using the Wilcoxon test with a probability of 0.05

Result

The intervention activities in the following research were compiled based on the model formed by providing Health Education through training to women of childbearing age to improve the women ‘decision to do early detection of cervical cancer with the VIA test. The number of samples obtained 35 utsamples in the treatment group and 35 samples in the control group, so that 70 respondents were obtained.

To analyze the difference in mean and standard deviation before and after treatment in each intervention and control group, a statistical analysis was performed using the Wilcoxon test with a probability of 0.05. The results of the analysis could be seen in the following table.
Table 1. Paired samples statistics research on the decision of women to do early detection of cervical cancer with VIA test in the treatment group in Pematangsiantar.

<table>
<thead>
<tr>
<th>Variable differences in woman’s decision to make early detection of cervical cancer</th>
<th>N</th>
<th>Mean</th>
<th>Difference pre-post</th>
<th>Std. Deviation</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre problem recognition</td>
<td>35</td>
<td>3,157</td>
<td>0,529</td>
<td>0,566</td>
<td>0,000</td>
</tr>
<tr>
<td>Pos problem recognition</td>
<td>35</td>
<td>3,686</td>
<td></td>
<td>0,455</td>
<td></td>
</tr>
<tr>
<td>Pre information search</td>
<td>35</td>
<td>3,086</td>
<td>0,628</td>
<td>0,562</td>
<td>0,000</td>
</tr>
<tr>
<td>Pos information search</td>
<td>35</td>
<td>3,714</td>
<td></td>
<td>0,369</td>
<td></td>
</tr>
<tr>
<td>Pre alternative evaluation</td>
<td>35</td>
<td>3,129</td>
<td>0,6</td>
<td>0,547</td>
<td>0,000</td>
</tr>
<tr>
<td>Pos alternative evaluation</td>
<td>35</td>
<td>3,729</td>
<td></td>
<td>0,408</td>
<td></td>
</tr>
<tr>
<td>Pre early detection</td>
<td>35</td>
<td>3,029</td>
<td>0,757</td>
<td>0,652</td>
<td>0,000</td>
</tr>
<tr>
<td>Pos early detection</td>
<td>35</td>
<td>3,786</td>
<td></td>
<td>0,389</td>
<td></td>
</tr>
<tr>
<td>Pre behavior after deciding</td>
<td>35</td>
<td>2,986</td>
<td>0,117</td>
<td>0,535</td>
<td>0,000</td>
</tr>
<tr>
<td>Pos behavior after deciding</td>
<td>35</td>
<td>3,757</td>
<td></td>
<td>0,390</td>
<td></td>
</tr>
<tr>
<td>Pre total intervention</td>
<td>35</td>
<td>15,387</td>
<td>3,285</td>
<td>2,862</td>
<td>0,000</td>
</tr>
<tr>
<td>Pos total intervention</td>
<td>35</td>
<td>18,672</td>
<td></td>
<td>2,011</td>
<td></td>
</tr>
</tbody>
</table>

In table 1. above Paired samples statistics The difference in the value of the pre and post test was seen from the difference in the mean value between the pre and post test and there was an increase in the value of the post test. The difference test results were significant between the pre and post test, the value of p = 0,000 (p <0.05)

Table 2. Paired samples statistics research on the decision of women of childbearing age to do early detection of cervical cancer with VIA test in the control group in Pematangsiantar.

<table>
<thead>
<tr>
<th>The variable difference in the decision of woman for early detection of cervical cancer</th>
<th>N</th>
<th>Mean</th>
<th>Difference pre-post</th>
<th>Std. Deviation</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre problem recognition</td>
<td>35</td>
<td>3,343</td>
<td>0,2</td>
<td>0,553</td>
<td>0,164</td>
</tr>
<tr>
<td>Pos problem recognition</td>
<td>35</td>
<td>3,543</td>
<td></td>
<td>0,475</td>
<td></td>
</tr>
<tr>
<td>Pre information search</td>
<td>35</td>
<td>3,343</td>
<td>-0,273</td>
<td>0,627</td>
<td>0,207</td>
</tr>
<tr>
<td>Pos information search</td>
<td>35</td>
<td>3,157</td>
<td></td>
<td>0,455</td>
<td></td>
</tr>
<tr>
<td>Pre alternative evaluation</td>
<td>35</td>
<td>3,000</td>
<td>-0,1</td>
<td>0,421</td>
<td>0,767</td>
</tr>
<tr>
<td>Pos alternative evaluation</td>
<td>35</td>
<td>2,900</td>
<td></td>
<td>0,930</td>
<td></td>
</tr>
<tr>
<td>Pre early detection</td>
<td>35</td>
<td>3,171</td>
<td>-0,043</td>
<td>0,321</td>
<td>0,631</td>
</tr>
<tr>
<td>Pos early detection</td>
<td>35</td>
<td>3,214</td>
<td></td>
<td>0,474</td>
<td></td>
</tr>
<tr>
<td>Pre behavior after deciding</td>
<td>35</td>
<td>3,200</td>
<td>0</td>
<td>0,584</td>
<td>0,972</td>
</tr>
<tr>
<td>Pos behavior after deciding</td>
<td>35</td>
<td>3,200</td>
<td></td>
<td>0,558</td>
<td></td>
</tr>
<tr>
<td>Pre total control</td>
<td>35</td>
<td>16,057</td>
<td>-0,083</td>
<td>2,506</td>
<td>2,741</td>
</tr>
<tr>
<td>Pos total control</td>
<td>35</td>
<td>16,014</td>
<td></td>
<td>2,874</td>
<td></td>
</tr>
</tbody>
</table>
In table 2, it showed that the Wilcoxon test results in the control group, there are differences in the mean values in the pre and post test. But the difference in the mean value between the pre and post test, there is no increase in the value of the post test. The results of the difference test were not significant between the pre and post test, the value of \( p > 0.05 \).

Table 3. Independent samples statistics research on woman of childbearing age’s decision to do early detection of cervical cancer with VIA test on the results of the treatment and control group test posts in Pematangsiantar.

<table>
<thead>
<tr>
<th>Variable differences in woman’s decision to make early detection of cervical cancer</th>
<th>N</th>
<th>Mean rank</th>
<th>Difference</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Problem recognition (pos control)</td>
<td>35</td>
<td>30,46</td>
<td>10,08</td>
<td>0,016</td>
</tr>
<tr>
<td>Problem recognition (pos treatment)</td>
<td>35</td>
<td>40,54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>information search (pos control)</td>
<td>35</td>
<td>27,36</td>
<td>16,28</td>
<td>0,000</td>
</tr>
<tr>
<td>information search (pos treatment)</td>
<td>35</td>
<td>43,64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alternative evaluation (pos control)</td>
<td>35</td>
<td>25,09</td>
<td>20,82</td>
<td>0,000</td>
</tr>
<tr>
<td>Alternative evaluation (pos treatment)</td>
<td>35</td>
<td>45,91</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do early detection (pos control)</td>
<td>35</td>
<td>25,14</td>
<td>20,72</td>
<td>0,000</td>
</tr>
<tr>
<td>Do early detection (pos treatment)</td>
<td>35</td>
<td>45,86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Behavior after deciding (pos control)</td>
<td>35</td>
<td>25,87</td>
<td>19,26</td>
<td>0,000</td>
</tr>
<tr>
<td>Behavior after deciding (pos treatment)</td>
<td>35</td>
<td>45,13</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In table 3, it showed that the Mann Whitney test results in the post control group and post treatment group, there were differences in mean rank values in the post control and post treatment. The difference in the value of the post control and treatment was seen from the difference in mean rank values, there was an increase in the mean value of the treatment post rank. The difference in problem recognition indicators in the control group with the treatment group obtained \( p = 0.016 \) (\( p < 0.05 \)), the difference in information search indicators in the control group with the treatment group obtained a value of \( p = 0.000 \) (\( p < 0.05 \)), different alternative evaluation indicators in the control group with the treatment group obtained \( p = 0.000 \) (\( p < 0.05 \)), the difference in indicators of early detection in the control group with the treatment group obtained \( p = 0.000 \) (\( p < 0.05 \)), differences in behavioral indicators after deciding in the control group with the treatment group obtained \( p = 0.000 \) (\( p < 0.05 \)).

Based on the results of the analysis in the table above, it can be concluded that the intervention model found can improve the decision of women to do early detection of cervical cancer with the VIA test.

The results of this study also found that from 35 women in the intervention group, after observing that in the second week after intervention, there were 20 women who decided to have early detection of cervical cancer with a VIA test and at the third week there were 10 people who decided to do early detection cervical cancer with VIA test. In the control group, after observation there were 5 women who decided to detect cervical cancer early with the VIA test.

Discussion

Based on the analysis of differences in decision of the women to do early detection of cervical cancer with VIA test between groups given intervention and groups not in the decision model intervention using non-
parametric test, and for Post test using the Wilcoxon test and for the Control vs test. The Mann Whitney Test treatment with the SPSS 22 program showed that the mean increase in the group given the intervention was greater than the control group. In the hypothesis test there were differences in the decisions of women between the groups given the intervention and the control group obtained significant values. Thus there was a change in the decision of women to do early detection of cervical cancer with an VIA test between the intervention group and the control group in Pematangsiantar.

The population in this study was less aware of cervical cancer. The increasing incidence of cancer and death, which was very alarming, caused by a lack of awareness of early detection of cervical cancer(12). The increase in cancer cases was more prevalent in developing countries than in developed countries(13) because cancer was detected most in the late stages in most developing countries, this poses several challenges in care, recovery and survival(14). Screening for early detection of cervical cancer in pematangsiantar was not optimal. This evidence-based information was an advocacy tool for policy making and the development of appropriate interventions. Health workers, especially in Puskesmas, need to improve their outreach services and provide health education to the community and increase timely information dissemination to the public regarding routine early detection of cervical cancer.

The study also showed a significant increase in the intervention group for cervical cancer. Woman of school understood more about each risk for cervical cancer. All participants agreed that women with complaints of public area should immediately check without delay. The preventive approach coupled with timely treatment was the only solution to overcome the burdensome cancer challenges. There was an urgent need to accelerate efforts to communicate accurate information, among different layers of society, about preventable cancers, especially cervical cancer, which was a leading cause of death in women(15).

To stimulate regular early detection among women, there must be aggressive health promotion interventions, designed to raise awareness and to improve the impression of cervical cancer in the community(16). Effective education and mass examinations are needed for successful cervical cancer screening programs in India because the creation of awareness and increased access to screening services was very important to reduce the burden of preventable cancer(17). This was consistent with research which found that women had poor knowledge about cervical cancer screening and lack of awareness of symptoms, risk factors and cervical cancer prevention screening. Importantly, the results of early detection would guide the management of conditions throughout life, including the decision-making process, where the individual would become an important part(15). Interventions to promote culturally sensitive public health programs designed to provide information and services across various age groups, levels of education, culture and social strata must be developed and implemented(18-20).

In conclusion, intervention with the decision model of women of childbearing age was able to change the decision to conduct an examination of early detection of cervical cancer to health services.

Conflicts of Interest: No potential conflict of interest relevant to this article was reported.

Ethics: The Ethical clearance of this study taken from the Commission on Health Research Ethics, Faculty of Public Health, Airlangga University.

Source of Funding: Thanks to the Overseas Seminar Assistance Program, Directorate General of Research and Development Strengthening, Kemenristekdikti.

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The Role of Wide Pore Drain in Successes of Spontaneous Closure of the Cysto-Biliary Fistula after Hydatid Disease of the Liver

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Abstract

This study to determine the role of wide pore use up in successes of spontaneous closure of cystic-biliary fistula after surgery of liver hydatid disease and decrease the postoperative morbidity. The study was conducted in AL-Zahra teaching hospital from May 2006 until May 2014. On 134 patients undergo surgery operation used for the liver hydatid cyst, complain of drain amount produced reliable with a biliary form and a bilirubin level elevated compared with serum bilirubin level after fifth postoperative day, patients were divided based on the presence of wide pore drain(64 patient) group A or classical drain(70 patient) group B. Sex, age, length of hospital stays, drain output, postoperative intervention relationship between cyst diameter and time for fistula to close spontaneously recurrence, and mortality were compared among both group. In group A, meantime for spontaneous fistula closure was 12.3 days, no recurrence or death reported, while in group B, meantime for spontaneous fistula closure was 17 days. There were three patients(4.3%) recurrence after one year follows up. There was one old female patient (1.4%) died.

Key words: Cysto billiary fistula CBF, Hydatid Cyst HC, Echinococcus Granulosis EG, Echinococcus Multilocularis EM, Wide Pore Drain WPD.

Introduction

Hydatid cyst is one of the oldest known diseases in humans. It was first illustrated in the Talmud as a result of a bladder filled with water and Hippocrates (379 BC) illustrated it as a liver filled with water. Hydatid disease is an epidemic disease in Iraq, and many Mediterranean countries and lead to disasters in health, economic and social. Iraq now is one of the most epidemic areas of this disease. Hydatid cyst is a worldwide infection caused by the larva’s state of parasite.

It belongs to the category of tapeworms named Echinococcus⁴. The areas where sheep and cattle breeding are the most epidemic in the world with parasitic worms especially South Australia, New Zealand, the northern part of Africa and some South American countries, as well human infections occur regularly in the European continent, Siberia, Mongolia, northern China, southern Japan, Vietnam, Philippines, Syria, Lebanon, Iraq, and Saudi Arabia⁵. Hydatid disease is a health, social and economic problem in the countries of the Eastern Sea Region.

The prevalence of infection is common in these areas because dogs are widely used in the care of cattle and sheep and have direct contact with humans, which leads to the continuation of the chain of infection which includes dogs and sheep as well as cattle, camels, goats and other animals (carnivores)⁷. In Iraq, the disease is epidemic and is a serious health problem, especially in the central regions between the Euphrates and the Tigris, these areas are inhabited by many producing farmers for grains and breeders for sheep as well as the presence of catalysts for the spread of hydatid disease such as lack of cleanliness and the presence of the middle host ⁸.

Female was higher than male patients ⁹, the main surgical occurrence was present in the 41–50 years age ¹⁰. ¹² The liver in males and females was more frequent site concerned than further organs and lung is next ²⁷. Since a best management, surgery was considered to be not dangerous, on the other hand minor and major problems speeds were wrote 32.2% and 25.0%, correspondingly, with a transience rate of 0.71%⁴, cystobiliary statement is the majority frequent
and difficult problem\textsuperscript{13} although these communication that under high pressure, may result in biliary obstruction, fistula, infection cholangitis, elevated bilirubin, elevated ALP levels and secondary biliary cirrhosis\textsuperscript{18} \textsuperscript{16} \textsuperscript{17} but the majority communication are occult in environment, and patients could stay clinically silent in the majority cases, with no any specific conclusion being noticed in the pre-operative develop and just being exposed through or next surgery\textsuperscript{18}.

**Material and Method**

Between May 2006 and May 2014, five hundred and one persons undergo surgery for liver hydatid cyst in Al-Zahra teaching hospital and Alkarama hospital in Al-Kut-Iraq. All persons undergo a complete blood cell (CBC) count and tests of liver function (LFT), fasting blood sugar (FBS), an abdominal ultrasonographic exam, chest radiography, were performed for all persons. Patients with gall stone, common bile duct stone and jaundice were excluded from the study.

The type of surgical procedure performed was laparotomy, packing the operative field with sponges soaked in 10% povidone iodine as scolicidal and injection of 10% povidone iodine to the cyst cavity, partial cystectomy with sclerotics and endocyst removal leaving pericyst and washing with 10% povidone iodine swab then tube drain inserted in the cyst and closed, there are two type of drain wide pore drain 32f-36f and classical drain 22f-26f, we use one drain for each patient randomly. Anyone from those five hundred and one patients complain of drain production reliable with a biliary form and bilirubin level upper than plasma bilirubin level after fifth postoperative day were included in this study and they are 134 patients. Persons were divided based on the presence of wide pore drain 32f-36f (64 patient) group A or classical drain 22f-26f (70 patient) group B.

Postoperative follow up of both group were done and a comparative study regarding, Sex, Age, length of hospital stays, drain output, postoperative intervention, relation ship between Cyst diameter and time for fistula to close spontaneously recurrence, and mortality were compared among both group. non compliance patient and patient with bad follow up were excluded from study.

**Finding**

The 64 patients (group A) 10 (15.6%) were male, and 54 (84.4%) were female. The median age was 42 years (range 16-74 years). The mean hospitalization time was 7 days, the mean daily drain output was 250 ml. Intervention need in, two female (3.1%) patient as catheter placement under USG regulation to control the biliary collection due to slipping of drain. The cyst diameter ranged from 5 cm to 20 cm (median, 9.0 cm). Mean time for spontaneous fistula closure was 12.3 days, no recurrence or death reported in this group. Of the 70 patients (group B), 15 (21.4%) were male, and 55 (78.6%) were female. The median age was 46 years (range 19-76 years). The mean hospitalization time was 9 days, the mean daily drain output was 450 ml. Intervention need in 5 patients (7.1%) four female and one male required catheter placement under USG guidance to control the biliary collections that were not adequately drained and obstructed. The median cyst diameter was 9.5 cm, mean time for spontaneous fistula closure was 17 days. There were three patients (4.3%) 2 female and one male recurrence after one year follow up. There was one old female patient (1.4%) deaths in this group due to biliary collections with subsequent sepsis. A comparative study regarding sex, age, length of hospital stays, postoperative intervention, relationship between cyst diameter and mortality were compared among both group and shown in the figures and tables below.
The relation between cyst diameter and median time for closure of fistula study found that larger cyst in group A closed earlier than middle and even small cyst while in group B study found that the time was proportional to the cyst size.

![Figure (2): The relation between cyst diameter and median time for closure of fistula in group A and B](image)

Table (2): Time for spontaneous fistula closure in group A and B.

<table>
<thead>
<tr>
<th>days</th>
<th>A</th>
<th>B</th>
<th>Total No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-10</td>
<td>4(6.3)</td>
<td>6(8.6)</td>
<td>10(7.5)</td>
</tr>
<tr>
<td>10-15</td>
<td>23(35.9)</td>
<td>13(18.6)</td>
<td>36(26.9)</td>
</tr>
<tr>
<td>15-20</td>
<td>14(21.9)</td>
<td>6(8.6)</td>
<td>20(14.9)</td>
</tr>
<tr>
<td>20-25</td>
<td>11(17.2)</td>
<td>8 (11.4)</td>
<td>19(14.2)</td>
</tr>
<tr>
<td>25-30</td>
<td>5(7.8)</td>
<td>16(22.9)</td>
<td>21 (15.7)</td>
</tr>
<tr>
<td>30-35</td>
<td>3(4.7)</td>
<td>12(17.1)</td>
<td>15(11.2)</td>
</tr>
<tr>
<td>35-40</td>
<td>2(3.1)</td>
<td>4(5.7)</td>
<td>6(4.5)</td>
</tr>
<tr>
<td>40-M 45</td>
<td>2(3.1)</td>
<td>5(7.1)</td>
<td>7(5.2)</td>
</tr>
<tr>
<td>Total</td>
<td>64(100)</td>
<td>70(100)</td>
<td>134(100)</td>
</tr>
</tbody>
</table>
Discussion

Postoperative hydatid biliary communication could effect in many morbidity and mortality\textsuperscript{19}, hydatid fistulae were described to be high resistant and not often stopped spontaneously. In spite of pre-operative and operative attempt to avoid this problem, most communication are occult in nature and appear after surgery.

In our series, postoperative cystic-biliary fistula occurred in 134 (26.7%) patients compared to 31% reported by Langer et al\textsuperscript{1-4}, women was affected more than men, 109 patients (81.3%) and 25 (18.7%) respectively, this confirming that most of hydatid cysts states between the housewives that present by many other researchers\textsuperscript{8-20}, on the other hand, elevated occurrence of the disease between housewives could be because of their close contact with infected dogs and most of them were from rural and agriculture area. The highest median age prevalence was 42 years in group (A) and 46 years in group (B), it is usually conventional that the majority of hydatid cysts are obtained in childhood, but could get several years to obvious themselves as harmful lesions\textsuperscript{25} hospital continue was more in group (B) compared with group A (9 vs 7 days) due to fever and wound infection in group (B), whilst the lowest was with group (A) indicating that wide pore drain is safer than classical drain and smooth postoperative days. Kemal et al reported anaphylactic shock and high fever and cholangitis and morbidity up to 10% in persons with liver hydatid disease\textsuperscript{21}.

The mean daily drain output was higher in group (B) compared with group (A) (450 ml vs 250 ml) Zeybek N et al show a postoperative biliary drainage volume less than 100 ml selected the only important forecaster of impulsive conclusion\textsuperscript{22}. The intervention need in group (B) was 7.1% (5) patients (%4 female and one male requisite catheter site under USG control to manage the biliary collection that were not sufficiently drained and obstructed while 3.2% in (A) as two female patient need catheter site under USG control to manage the biliary collections due to slipping of drain, drain slipping occur due to large drain diameter and can be solved by frequent suturing of drain.

Drain slipping and unavailability of wide pore drain are the most two deteriorating factor that we in faces in our study Some study intervention with ERCP sphincterotomy and the puncture, aspiration, injection and re aspiration (PAIR) procedure\textsuperscript{23} In This study about 35.9% of fistula closed within 10-15 days in group (A) while only 26.9% in group (B), and 4.7% only need more than one month to close in group (A) while 17.1% in B. The relation between cyst diameter and median time for closure of fistula we found that larger cyst in group (A) closed earlier than middle and even small cyst while in group (B) we found that the time was proportional to the cyst size, this can be attributed to Bernoulli’s principle and Poiseuille’s law on our drain and use 36 French instead of 22 French there will be more flow of drainage and decrease in intracystic pressure and decrease in surface tension this will lead to early fistula closure,
and decrease the postoperative morbidity, also can be attributed to that high intraabdominal pressure on rapid empty cyst cavity lead to collapsing the cyst early and blockage of fistula.

The reappearance speed was 4.3% in group (B), but zero in group (A) more than the similar time of ordinary postoperative follow up, this might be considered as evidence that wide pore drain is much more effective than classical drain these attributed to that communicate through the biliary structure, resultant in an increasing disease of the cyst contents and bacterial super infection could destroy the parasite and wide pore drain allow good drainage of missed small scoliosis the recurrence rate in most literature did not exceed 10%.²⁴ In this study, Postoperative mortality was 1.4% in group (B) and zero in group (A). Which near the literature that stated as 1-3%,²⁴ so redaction in postoperative mortality also can be achieved.

**Conclusion**

According to Bernoulli’s principle and Poiseuille’s law on our drain and use 36 French instead of 22 French there will be more flow of drainage and decrease in intracystic pressure and decrease in surface tension this will lead to early fistula closure, and decrease the postoperative morbidity and mortality.

Security permits: clearance was taken from a government hospital in Wasit province with the knowledge of the government administration and patients.

Ethical statement of the subject of research came with the approval of the doctors supervising the condition of their patients in addition to the reviewers to follow up their healthy condition throughout the research period.

Research funding was done by the researcher himself and without any government funding.

**References**


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Causes of Emergency Hysterectomy in AL-Fallujah Maternity & Children Teaching Hospital

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Abstract

Objective is to detect hysterectomy and maternal morbidity and mortality that occurs in emergency hysterectomy in secondary hospital. This retrospective study of 9 patients done for obstetrics indications from January 2018 to January 2019, in department of Gynecology and obstetrics Al-Fallujah teaching hospital. patients and methods 9 cases at this study period underwent emergency hysterectomy, data collected from hospital record their personal data, chief compliant, past obstetric, cause of hysterectomy and complication was recorded. Result There were 9 cases emergency hysterectomies done for obstetric condition. The indications and surgical complications was studied.

Conclusion: The emergency hysterectomy is mandatory to save life of patients. The outcome is depending on the decision time and the expert of the judgment because any delay may cause increase mortality and morbidity

Keywords: Emergency; hysterectomy; AL-Fallujah Teaching Hospital.

Introduction

Emergency obstetric hysterectomy mean uterine removal at any time of cesarean section, after caesarian section, after normal delivery or in perpartum period in order to save the patients. (1)

Horatio Storer did ported the first operation at 1898, but it was un successful. After seven years Eduardo Porro did the first successful operation with subtotal hysterectomy it is called “PORRO OPERATION” (2)

In developed countries, hysterectomy is done mainly for sterilization and uterus tumors. In developing world it is done mainly when there is frailer of controlling of hemorrhage after delivery. (3) The rate ranges from 0.4 to 2.5 per 1000 births and has risen significantly during the past few decades. Most of this increase is attributed to the increasing rates of cesarean delivery and its associated complications in subsequent pregnancy (4).

Hysterectomies, approximately to One half to two thirds are total, whereas the remaining cases are supra cervical (5).

Table 1: Some Indications for Peripartum Hysterectomy (7)

<table>
<thead>
<tr>
<th>1-Uterine atony</th>
<th>2-Abnormal placentation</th>
<th>-Bleeding</th>
<th>-Accrete syndromes</th>
<th>3-Uterine extension</th>
<th>4-Uterine rupture</th>
<th>5-Cervical laceration</th>
<th>6-Postpartum uterine infection</th>
<th>7-Leiomyoma</th>
<th>8-Invasive cervical cancer</th>
<th>9-Ovarian neoplasia</th>
</tr>
</thead>
</table>

Hysterectomy mainly done due to uterine atony that occur in 1:1000 delivery (5) Post partum cause
of hysterectomy occurs if the patients have previous caesarian section and the placenta implant over the previous scar(6) These as well as other less frequent indications are found in table (1) For example, large leiomyoma may preclude satisfactory hysterotomy closure and necessitate Hysterectomy . Or, postpartum infectious morbidity from an infected , necrotic uterus will prompt uterine removal for recovery (7).

Uterine atony is failure of contraction of the muscles after separation of the placenta that cause sever bleeding .The majority of PPH cases (75-80%) are due to uterine atony .

The factors predisposing to postpartum uterine atony are listed in table (2).

**Table (2) FACTORS PREDISPOSING TO POSTPARTUM UTERINE ATONY (8)**

<table>
<thead>
<tr>
<th>Previous postpartum hemorrhage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prolonged labor</td>
</tr>
<tr>
<td>Grand multiparity (a parity of 5 or more)</td>
</tr>
<tr>
<td>Conditions that Overdistended uterus</td>
</tr>
<tr>
<td>Multiple gestations</td>
</tr>
<tr>
<td>Polyhydramnios</td>
</tr>
<tr>
<td>Fetal macrosomia</td>
</tr>
<tr>
<td>Oxytocic augmentation of labor</td>
</tr>
<tr>
<td>Precipitous labor (one lasting &lt;3 hr)</td>
</tr>
<tr>
<td>using of Magnesium sulfate in preeclampsia</td>
</tr>
<tr>
<td>Chorioamnionitis</td>
</tr>
<tr>
<td>Using of halogenated anesthetic drugs</td>
</tr>
<tr>
<td>Uterine leiomyomata</td>
</tr>
<tr>
<td>Vitamin D deficiency</td>
</tr>
<tr>
<td>Genetic and epigenetic factors (maternal, environmental, and fetal)</td>
</tr>
</tbody>
</table>

Most of the blood loss due to uterine atony occurs from the myometrial spiral arterioles and decidual veins that previously supplied and drained the intervillous spaces of the placenta. As the contractions of the partially empty uterus , hemorrhage happens and continue and stopped if the uterine muscle contrac and press the blood vasculature and cause ligation physiologically(8). The hemorrhage occurs also when there is rupture of uterus especially when there is a rupture scar ,from manipulation ,trauma ,congenital anomaly of the uterus or may be occur spontaneously.

Rupture also occur in abnormal delivery, operated labor and placenta accrete Placenta accrete is abnormal attachment between placenta and lining of the uterus with loss of decidua basalis. the incidence of placenta accrete occur in about 3:1000 . Other important risk factor are previous cesarean section of the uterus .(9)

Placenta percreta causes invasion of urinary bladder that presented with hematuria . Placenta accrete mostly diagnosed antenatally by radiological facilities (ultrasound is the preferred ). Placenta previa mean presence of the tissue of placenta on or closely to the internal cervical os .(10)

There are two types of placenta previa in recent classification .The true placenta previa in which placenta covers the internal os completely .The second type is low lying placenta in which the placental tissue presented within two cm away from cervical os and doesn’t cover it . The incidence of placenta previa is about 1 : 200 births. The placenta previa occur upto 6% in second trimester and cause painless vaginal bleeding . The bleeding occur at least once in about 70% to 80% of the patients .(11). Risk factor are Increasing parity, age, race , Cigarette smoking and cocaine use , Prior placenta previa , Prior uterine surgery and prior cesarean delivery (10).

Logically , the risk factors for peripartum hysterectomy mirror the risks of these indicated complications Major complications of peripartum hysterectomy include increased blood loss and greater risk of urinary tract damage Blood loss is usually appreciable because hysterectomy is being performed for hemorrhage that frequently is torrential , and the procedure itself is associated with substantial blood loss (7).

The maternal death mainly caused by shock ,DIC ,complication of operation and post operation complication.
The hysterectomy had been reduced due to advanced method of management of the labor, use of antibiotics, oxytocin and prostaglandins. It is also decreased by use of internal iliac artery ligation (11).

After anticipated or planned cesarean hysterectomy, there are lower rates of blood loss, less need for blood transfusions and fewer urinary tract complications compared with emergent procedures (12).

This study attempts to highlight the extent of availability and utilization of antenatal services, identify avoidable factors and stress the need to organize health care services so as to improve maternal and fetal outcome.

The decision about Hysterectomy should be made by an experienced consultant clinician (preferably after discussion with a second experienced consultant clinician) and the procedure should be carried out by a surgeon who is experienced in carrying out hysterectomy. Do not delay hysterectomy until the woman is in extremis or while less definitive procedures with which the surgeon has little experience are attempted. Resort to hysterectomy sooner rather than later (especially in cases of placenta accrete or uterine rupture). Subtotal hysterectomy is the operation of choice in many instances of PPH requiring hysterectomy unless there is trauma to the cervix or lower segment (13).

Aim of this Study is to determine the indications of obstetric hysterectomy, maternal morbidity and maternal mortality associated with emergency obstetric hysterectomy at a secondary hospital, Department of Gynecology and Obstetrics.

Patients and methods

We retrospectively analyzed by case series a total of 9 women who underwent emergency peripartum hysterectomy between January 2018 and January 2019 at Al-Fallujah teaching hospital, Department of Obstetrics. The data was collected from the hospital records. All women included in this study underwent hysterectomy within first hour of delivery.

The medical record sheets of all identified women were reviewed regarding age, occupation, geographic area, blood group, parity, miscarriage, multiple or single pregnancy, chief complaint at admission, past obstetric history which include: (previous e/s, myomectomy, hysterotomy, placenta previa or and accrete, any previous pelvic surgery), causes of hysterectomy which include: (placenta previa or and accrete, rupture uterus, H.mole, and uterine atony) and complication in operative room which include: (bladder injury, bowel injury, and ureteric injury).

Results

Case Finding:

Case 1: A 30 years old female, housewife from rural area o- blood group P5M0 presented to ER with abdominal pain and slight vaginal bleeding for 30 min before admission. The pain started after few minutes from receiving IM injection of oxytocin at midwife house. After examination she diagnosed as uterine rupture and she underwent emergency C/S in operative room. Surgeon decided to do emergency hysterectomy; The surgery was associated with ureteric injury.

Case 2: A 39 years old female, housewife from Urban area o- blood group P6M0 single pregnancy admitted as elective C/S and she was known case of placental abruption. She had previous 3 C/S and other past obstetric history was negative. The patient prepared for surgery and at operative room surgeon discovered multiple adhesion and bleeding started so surgeon decided to do emergency hysterectomy; The surgery was associated with bladder injury.

Case 3: A 35 years old female, housewife from rural area A+ blood group P5M0 single pregnancy presented to ER with loss of consciousness. Patient lost her consciousness during labour at midwife; she received IM injection of oxytocin at midwife house. She had previous 3 C/S and other past obstetrical history was negative. Examination done and she diagnosed as uterine rupture so she underwent emergency C/S and in operative room surgeon decided to do emergency hysterectomy; The surgery was associated with bladder injury.

Case 4: A 38 years old female, housewife from Urban area A+ blood group P6M0 single pregnancy presented to ER with vaginal bleeding. She had previous 6 C/S other past obstetrical history was negative and she underwent emergency C/S, and in operative room there was placenta accrete with multiple adhesion and severe bleeding so patient underwent emergency hysterectomy; The surgery was associated with bladder injury.
Case 5: A 29 years old female, housewife from Urban area B- blood group P6M1 single pregnancy admitted for elective C/S. She had 3 previous C/S and other past obstetric history was negative. The patient prepared for surgery during operation the surgeon discovered there is placenta accrete and multiple adhesion, so decided to do emergency hysterectomy; The surgery was associated with bladder injury.

Case 6: A 36 years old female, housewife from rural area B+ blood group P8M0 single pregnancy presented to ER with abdominal pain. She had 3 previous c/s and other past obstetrical history was negative. The patient prepared for surgery, during operation the surgeon discovered there was placenta accrete and multiple adhesion. Emergency hysterectomy was done; the surgery was associated with bladder injury.

Case 7: A 30 years old female, housewife from Aruban area O- blood group P5M0 single pregnancy presented to ER with vaginal bleeding. She had 4 previous c/s and other past obstetrical history was negative. The patient prepared for surgery during operation surgeon discovered there was placenta accrete with multiple adhesion there was severe bleeding so decided to do emergency hysterectomy; The surgery was passed without complication.

Case 8: A 30 years old female, housewife from Urban area O+ blood group P5M0 single pregnancy presented to ER with vaginal bleeding. She had 1 previous c/s and other past obstetrical history was negative after examination she diagnosed as uterine rupture and she underwent emergency C/S during surgery there was severe bleeding started bleeding and decided to do emergency hysterectomy; The surgery was passed without complication.

Case 9: A 21 years old female, housewife from Urban area O+ blood group P1M0 single pregnancy presented to ER with abdominal pain for 30 min before admission pain colic in nature radiated to the back associated with gash of watery vaginal discharge. Past obstetric history was negative. Examination done and she referred to vaginal delivery room after 4 hours she delivered Baby and after less than 30 min delivered the placenta and bleeding started and not stopped by conservative measures so referred to operative room also all available measure done without benefit, so the surgeon decided to do Emergency hysterectomy, The surgery was passed without complications.

Discussion

Peripartum hysterectomy is a major surgical intervention usually done as an urgent procedure that associated with significant maternal and fetal complications. (14)

Nine cases of emergency hysterectomy in the study period from January 2018 to January 2019 at Al-Fallujah teaching hospital, Department of Gynecology. Eight cases out of 9 that underwent obstetric hysterectomies were multigravida.

Most common indication of hysterectomy in this study was placenta accrete. 5 from 9 cases underwent emergency hysterectomy due to placenta accrete. All patients had previous c/s.

The second most common was ruptured uterus 3 from 9 cases underwent emergency hysterectomy due to ruptured uterus. And one case due to uterine atony. The age of females ranging from 21 to 39 year and all of them were housewife, most of them from Aruban area.

From case series three patients were diagnosed with ruptured uterus they went to midwife and received IM injection of oxytocin and two of them had previous c/s. and two of them from rural area. Intraoperative records revealed 5 of these cases had bladder injury, 1 ureteric injury and 3 of them surgeries ended without complications.

Conclusion

1. Peripartum hysterectomy is the “near miss event in both developed and developing countries. It has been described as one of the riskiest and most dramatic operations in modern obstetrics. It is therefore associated with significant maternal mortality and morbidity. Its prevention is the foremost goal in obstetrics.

2. Identification of high risk cases, early referral, timely performance of caesarean section careful monitoring and resort to conservative procedures can reduce the near miss event.

3. Most difficult situation is doing hysterectomy in primigravida or in patients with no living children. So it should be performed judiciously weighing the need to sacrifice the obstetric future of the patient in favor of patient’s life.
4. Special provision of blood transfusion, dialysis facilities and good ventilatory support is necessary round the clock.

5. Availability of multidisciplinary team involving an experienced Obstetrician, anesthetist, urologist, interventional radiologist and a physician round the clock is necessary.

6. Availability of communications and transport facilities for these emergency patients are required. Provision of emergency ambulance facility services provided by the government has played a huge role in quicker access for health care facilities.

7. Further such measures will help in reducing maternal and perinatal morbidity in emergency peripartum.

**Conflict of Interest:** non

**Source of Findings:** self findings.

**Ethical Clearance:** This research was carried out with the patient’s verbal and hospital approval before the cases was taken.

**References**


The Immunological Study of Salmonella Infantis in white Mice Immunized with Killed Antigen

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¹Department of Medical Laboratory Techniques, Kut Technical Institute, Middle Technical University, Baghdad, Iraq, ²Department of Nursing, Institute of Medical Technology, Middle Technical University, Baghdad, Iraq

Abstract

The present study was carried out to investigate the effect of formalin-killed antigen for Sallmonellainfantis in vivo with the study of cellular immune response through the study of cytokines.Sallmonellainfantis were isolated and diagnosed from local milk products (milk and cheese). 74 milk samples and 53 cheese samples were taken. The media were used general and special culture media as well as biochemical tests were used to diagnose the isolates and the diagnosis was confirmed by using API 20.

Sallmonellainfantis antigens were used systemically. 50 Swiss white mice of both sexes were randomly divided into three groups. The first group prevented (20 mice) with the bacterial antigen killed by formalin in a dose (0.3) I.P for each animal. The group was injected with a booster dose and the same first immunization dose two weeks after the first dose. The second group (20 mice) and the third group (10 mice) were counted as positive and negative control groups respectively.

The first group and the second group received a dose of 0.3 ml of stuck S.infantis containing (1×10⁸ live cells / ml) I.P, while the third group injected 0.3 ml of neutral phosphate buffer solution under the skin. The results showed that 4 animals from the first group were killed and all the remaining animals from the first group were killed 20 days after the challenge dose and serum was taken to measure cellular immunity. The results of serological tests showed that the level of (IL-4 and IL-6) were (38.48 ± 2.1 and 31.81, 3.01) respectively, as well as the results of measurement of concentration (INF gamma and TNF-α) were (401 ± 2.12 and 173.421 ± 3.11) respectively. We conclude from the study that there was a high percentage of S.infantis isolated from milk products as well as we conclude that the formalinS.infantiskilledantigen partially protected against the infection.

Key Words: Sallmonellainfantis, killed Antigen , IL-4, IL-6, INF gamma, TNF-α.

Introduction

Non-typhoidal Salmonella enterica (NTS) are considered to be one of the main common causes food borne infection worldwide that effect on humans (1). Salmonella Infantis is the most recurrence strain in numerous countries, involving Asian countries. detection and isolate of S. Infantis from animals, humans and vegetables (2,3). when salmonellosis causes infect of animals, due to increase in death -rate in infected animal leads to economic loss for countries, these Infected animals act as a source of infection that during to direct or indirect contact injury to humans (4). The humans and animals infected with salmonella contaminate the environment and nutrition at most by feces (5) also Salmonella colonizes at most in the intestinal tract (6). The dairy products and Milk, especially poorly pasteurized, are among the possible causes of the transmission of many pathogens to humans through food (7). The feces of infected cattle, infected udder, milking equipment, contaminated skin, feed and from milkers considered as main sources of contaminate raw milk, causes salmonellosis and other pathogen (8, 9, 10).

The present study was carried out to investigate the prevalence of Salmonella infant in milk and cheese, and revealout the serotypes of the Salmonella isolates.
Materials and Method

Sample collection:

Dairy products (milk and cheese) samples were collected from local supermarkets in Kut city. The samples were collected during the period from January to April 2019. Samples were rapidly put in selenite broth containing sterile tubes and transmitted by ice box during 2 hour to the laboratory of bacteriology in pathological analysis department in Kut Technical Institute.

Bacterial Isolation:

All samples collected were cultured onto MacConky agar and incubated at 37°C for 24-48 hour. The growing colonies were examined by naked eye concerning their color, shape and size, sub-cultured onto selective media such as Eosin Metheline blue, SS and XLD agar and incubated at 37°C for 24 hour. Method of culturing was done according to (11).

Bacterial Identification:

The colonies were examined by eye according to their color, shape, size, the Gram stain was done in addition to biochemical tests including: Catalase test, Oxidase test, Lactose fermentation, Urase test, Indole test, Citrate utilization test these test were done according to (12), then confirm diagnosis of the isolates by API 20E according to (13).

Challenge dose

The Salmonella Infantis was cultured, growth and purification on the media of this study, the live bacterial cell counted according to (14).

Antigens (killed Salmonella Infantis antigen):

The killed S. Infantis antigens were prepared according to (15).

Cytokine assessment:

Interleukin-4, Interleukin-6, INF gamma and TNFα Assay Procedure, The procedure is performed at room temperature according to manufacturer’s instructions (Boster’s – Korea).

Experimental design:

Fifty white mice were used (male and female), the ages (8-10 weeks) and their weight ranged between (25-30 gram), obtained from institute of sera and vaccines ministry of health, and reared in cages furnished clean sawdust, and fed concentrate feed during the duration of the experiment. The white mice were divided into three groups were first group includes 20 mice immunized inoculated with 0.3 ml of formalin killed antigen, two dose, 2 weeks intervals at day 30 post immunization, and it was inoculated I/P with 1X10^8 cfu/ML. The second group includes 20 mice was considered as positive control, it was inoculated I/P with as 1st group. Third group includes 10 mice was inoculated I/P with 0.3 ml of sterile normal saline and served as control negative group. At day 30 post infection, all animals were sacrificed and blood samples were collected for determine cell mediated immune response by measurement of cytokines including IL-4, IL-6, INF gamma and TNF-α.

Results

Bacterial isolation:

The results were revealed the percentage of bacterial isolation from dairy product (milk and cheese) as in table 1:

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>No. of sample</th>
<th>Positive isolation</th>
<th>Percentage of positive isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td>74</td>
<td>46</td>
<td>62.61%</td>
</tr>
<tr>
<td>Cheese</td>
<td>53</td>
<td>31</td>
<td>58.49%</td>
</tr>
<tr>
<td>Total</td>
<td>127</td>
<td>77</td>
<td>60.62%</td>
</tr>
</tbody>
</table>

Immune response

The cellular immune response

The results were showed that serum levels of IL-4 and IL-6 were 38.48±2.1 and 31.81±3.01 respectively as shown in table 2.
Table 2: Cytokine profile of Salmonella infantis in immunized mice post infection

<table>
<thead>
<tr>
<th>Groups</th>
<th>IL-4 (pg/ml) mean±SE</th>
<th>IL-6 (pg/ml) mean±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 immunized group</td>
<td>38.48±2.1</td>
<td>31.81±3.01</td>
</tr>
<tr>
<td>G3 negative group</td>
<td>3.5±0.36</td>
<td>2.1±0.45</td>
</tr>
</tbody>
</table>

The results were showed that serum levels of INF gamma (pg/ml) at 30 days and TNF-α (pg/ml) at 30 days were 401±2.12 and 173.421±3.11, respectively as shown in table 3.

Table 3: Mean values of serum levels of INF gamma in immunized mice post infection

<table>
<thead>
<tr>
<th>Groups</th>
<th>INF gamma (pg/ml) mean±SE at 30 days</th>
<th>TNF-α (pg/ml) mean±SE at 30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1 immunized group</td>
<td>401±2.12</td>
<td>173.421±3.11</td>
</tr>
<tr>
<td>G3 negative group</td>
<td>48±0.81</td>
<td>25.7±0.601</td>
</tr>
</tbody>
</table>

Discussion

The present study recorded highly percentage of bacterial isolated in milk and cheese (62,16 and 58,49)% respectively. This result could indicated that the food product (milk and cheese) were thought of a supply a microorganism infection of human once dangerous handling, particularly in Asian country, the individuals consumed great amount of diary product, these results agreement with The farm product and Milk, particularly poorly change integrity, are among the attainable causes of the transmission of the many pathogens to humans through food. The current study showed the immunized group with virulence salmonella infantis when infected with lethal dose the death only 4 mice from this group (20 mice) during post 48 hr lethal dose infection, these may be, the killed antigen give partially protection, these results agreement with (16,17) who showed this might be indicate that antigens stimulate response with partial protection potential that destroys some microorganism at website of immunisation however sizable amount of those organism reach to internal organs and proliferation but the body tried to localize of this organism by neoplasm reaction. While positive Control (non immunized group) when infected these animals with lethal dose, the death of all animals during 24 hr post lethal dose infection may indicated that, the S. infantis is highly virulence bacterium that overcome traditional defense mechanisms of the host and spread to internal organs cause bacteraemia and sepsicaemia that cause high mortality, this results are agreement with (18) UN agency showed that, non infectious disease enterobacteria is major causes of morbidity and mortality post dissemination infections.

These results of present study were revealed that serum levels of IL4 and IL6 in immunized group were (38.4±2.1 and 31.81±3.01) respectively. The cytokine profile in immunized mice were showed the killed bacteria as a good immunogenic antigen which stimulate immune response with an intrinsic immunologic adjuvant which can lead to enhancement off immune response and at the same time can immunosuppressed. The cells which affecting their function might be through regulation T-cell (19). At sites of microbial invasion, leukocyte and other cells send out distress signals by releasing cytokines or chemokines. These proinflammatory signals activate local cardio vascular endothelium (IL1, TNFα) to express selection molecules, increase expression of chemotactic molecules (IL1 and IL8) and activate leukocyte (IL1, IL6, IL8, IL12 and TNFα), these results agreement with (20,21).
The result of current study may be due to bacterial component which stimulate TLRs that mediated pro-inflammatory cytokines production such as TNFα, IL8 and IL6 which considered attraction of neutrophil, these results are agreement with (22). who showed that salmonella infection in animal models characterized by abundant neutrophils infiltration.

The our results of immunized mice were showed that serum levels of IFN gamma and TNFα were (401±2.12 and 173.421±3.11) respectively. These results showed decreasing bacterial load in the present study with stimulated cell mediated immune response may indicated that both innate and acquired immune response may indicated play important role in eradication of S. infantis infection, these results are agreement with (23) who recorded that innate immune system which included phagocytic cells and cytokines can controlled the low virulence of these pathogen required both innate and acquired immune response including TNFα, IFN gamma, IL12, NK cells, humeral and cellular immune response particularly in the previous immunized or exposure to sub lethal infection showed that both B and T cell play important role in the protection against salmonella infection (24).

These was few bacterial isolated from examined organs of immunized infection mice which all of them were death during first 24hr post infection with sever bacterial load in all examined organs, these results agreement with (25) who recorded that vaccinated mice provided Th1-cytokines (TNFα, IFN gamma and IL12) as well as salmonella specific antibodies that controlled challenge with virulence salmonella infection.

**Conflict of Interest:** Non

**Source of findings:** Self findings.

**Ethical Clearance:** Non

**References**


The Inhibition Effects of *Withania somnifera* leaves Extracts for Multi Drugs Resistance Bacterial Isolates

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**Abstract**

The continuous resistance of pathogenic bacteria to antibiotics and failure of treatment of human diseases cause by these micrograms in addition to important of *withania somniera* plant in herbs medicine the present study carried out to assessment the antibacterial effect of three types of leaf extract: acetone, methanol and water in different concentrations to four multidrug resistance pathogenic isolates were selected two Gram positive and two Gram negative, the result showed that all types of isolated were inhibited by leaf extracts whereas the acetone record the highest effect were the inhibition zone (millimeter) of growth of four bacteria isolates *pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Streptococcus pneumonia* reach 15, 14, 13 15 mm respectively at 100 mg/ml concentration of acetone, the current finding indicated that leaf extracts useful as a good natural alternative drug from antibiotics in treatment of numerous infectious diseases these result from both Gram positive and Gram negative bacteria.

**Key words:** *Withania somnifera*, leaf extracts, antibacterial effect.

**Introduction**

Pathogenic bacteria responsible for the serious diseases to human and important causes of morbidity and mortality worldwide, The continuous increase and development of pathogenic bacterial resistance to antibiotic according to different mechanisms of resistance, and due to random usage of antibiotic in human treatment and veterinary medicine (1), multidrug resistance (MDR) of bacteria cause reduction in the effectiveness and failure of the synthetic drugs to treatment the microbial infections either Gram negative or positive species (2) (3), in addition to high cost of antimicrobial manufacture so that the scientist carried several studies and researches’ to dissolve and to minimize the treatment problems to found alternative drugs from the natural source.

Worldwide reported the presence important compounds as constituents of medicinal plants and posses high activity against bacteria and considered natural, low cost, available sources use as drugs in treatment of microbial diseases. (4)

*Withania somnifera* from Solanceae family known Ashwagandha Indian ginseng winter cherry use in Ayurvedic folk medicine has potent effect to treatment several diseases as Arthritis stress, Sedative, cough, others (5). Leaves constitutes of *Withania somnifera* riches with withanolides, alkaloids, Steroidal lactones, tannin, flavonoids, other compounds (6). Several studies carried to detect the action of *Withania somnifera* extracts against the growth of pathogenic bacteria isolated from different clinical samples, Rizwana et al 2012 showed in their study of antibacterial affect of acetone, Methanol, Chloroform extracts of *Withania somnifera* the high inhibition effect of the extracts on the growth of seven species of human pathogenic bacteria (7).

*Withania somnifera* plant useful as drug in treatment of wounds, skin diseases, anti cancer, digestive system disorders, respiratory tracts infection (8,9). Antibacterial activity of leaf extracts at concentration 6.25 - 12.25 mg/ml give high growth inhibition of *Escherichia coli*, *Salmonella typhi*, *klebsiella pneumonia* *citrobacter freundii*, *pseudomonas aeruginosa* (10).

The objective of our current study was performed to focus the light on the potential of antibacterial effect of *Withania somnifera* leaf extracts against growth of four Multi Drugs resistance bacterial isolates.
Materials and Method

Bacterial Isolation & Identification:

The present was carried during January 2019 to April 2019 in Baquba city for isolation and identification of bacteria from pathogenic samples (urine, wounds swabs, nasal swab, Sputum). Each sample was inoculated on to the 50% sheep blood agar, MacConkey agar (without crystal violet), each plate were incubated overnight under aerobic condition at 37°C the identification of bacteria performed on the base of colony characteristics, morphology of bacteria (staining, shape and arrangement), and biochemical tests include (IMPIVC, Oxidase test, urease test, Coagulase and DNase test for *Staphylococcus aureus*, Catalase, Hemolysin test, Triple – sugar iron agar test, & confirmation of isolate identification performed by Vitek 2 Compact System (11).

Multi Drug Resistance (MDR) Isolates:

To detect the percentage of resistance of pathogenic bacterial isolates fourteen antibiotics were used include Gentamicin (10), Ampicillin (10), Tetracycline (30), Amikacin (10), levofloxacin (10), Ciprofloxacin (5), Trimethoprim – Sulfamethoxazole (25), Cefepime (30), Meropenem (10), Erythromycin (15), Pencillin G (10), Amoxicillin calvulanic acid (10), Vancomycin (30), Norfloxacin (10), using Kirby – Baure (Disc Diffusion method), suspension of bacteria with 1.5 x10 cell/ml were prepared in three replicates, leave the plate for 30 minutes in room temperature to allow the diffusion of the inhibition zone of each extract were recorded by 0.22 mm Millipore filter paper (12).

Four concentrations (25, 50, 75, 100 mg/ml) of acetone, methanol, water extracts were prepared by dissolved 1 gm of leaf powdered of each types of extract in 10 ml of acetone, methanol, distill water, then dilute to obtained the demand concentrations and sterilize by 0.22 mm Millipore filter paper (13).

*Withania somnifera* leaf Extract Activity

Agar well diffusion method were used to investigate the activity of leaf of *Withania somnifera* leaf extracts at concentrations 25, 50, 75, 100 mg/ml in three solvents (Acetone, Methanol, Water) against the growth of MDR isolates belongs to four pathogenic bacteria species. Colonies of isolates activate by subculture in brain heart infusion broth and incubate for 24 hours at 27°C at bacterial suspension with 5x10 cell/ml were used by compare with McFarland standard 0.5 solution, 100µl then spread by sterile cotton swabs on the surface of Muller- Hinton agar and on blood agar for *Streptococcus pneumoniae*, wells with 6mm diameter were makes by sterile cork bores and transport 50uL from 25, 50, 75,100mg/ml of plant extract to the wells each treatment performed in three replicates, leave the plate for 30 minute in room temperature to allow the diffusion of extract, the plates incubated for 24 hours at 37°C reach the inhibition zone of each extract were recorded by millimeter and calculated the mean of the zones from three replicates (14).

Results and Discussion

The result sensitivity of isolates belongs to four species of pathogenic bacteria against fourteen antibiotics identified from clinical samples showed the presence of several isolate has multidrug resistance( multi drug resistance isolate include two species of Gram negative *Pseudomonas aeruginosa* and...
Klebsiella pneumonia, and two species of gram positive Staphylococcus aureus and Streptococcus pneumoniae were selected to investigate the antibacterial activity of three types of Withania somnifera leaf extract. Our the finding revealed that multi drug resistance of each isolates, pseudo monas aeruginosa resistant to five antibiotics include Gentamicin, Amikacin, levofloxacin, Tetracycline, Ciprofloxacin, Klebsiella pneumonia resistant to four antibiotics: Amikacin, Trimethoprim – Sulfamethoxazole, Cefepime. The results of sensitivity of Gram positive isolates showed multidrug resistance to other groups of antibiotics Staphylococcus aureus resistant Gentamicin, Ampicillin, Tetracycline, Amikacin and Streptococcus pneumonia resistant to Meropenem, Erythromycin, Pencillin, Ampicillin, Tetracycline, Amoxicillin antibiotics.

Table (1):- Multidrug resistance of one selective pathogenic bacteria isolates

<table>
<thead>
<tr>
<th>Bacteria species</th>
<th>MDR isolates</th>
<th>Antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Source of isolate</td>
<td>Antibiotic number</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Nasal swab</td>
<td>4 Gentamicin, Ampicillin, Tetracycline, Amikacin</td>
</tr>
<tr>
<td>Psuedomonas aeroginosa</td>
<td>Burns infections (skin swab)</td>
<td>5 Gentamicin, Amikacin, levofloxacin, Tetracyclin, ciprofloxacin</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
<td>Urinary tract infections (urine)</td>
<td>4 Amikacin, Trimethoprim – Sulfamethoxazole, Cefepime</td>
</tr>
<tr>
<td>Streptococcus pneumonia</td>
<td>Pulmonary Pneumonia (sputum)</td>
<td>6 meropenem, Erythromycin, Penicillin, Ampicillin, Tetracycline, Amoxicillin-calvulanic acid</td>
</tr>
</tbody>
</table>

The presence of different clusters gens in specific sites on plasmids or chromosomes responsible for the antibiotic resistance of bacteria and the association between those genes and other gens increase the chance of bacteria survival and adaptation may be result to the presence of multiple resistance genes in the bacteria strains, in addition of genetic processes mainly conjugation which considered one of the major mechanisms conjugative plasmids for transmission of resistance elements between bacteria strains as class 1 integron which considered as a part of antibiotic resistance Island which & into different conjugation plasmids.

Anti bacterial effect of leaf extracts:

The result in tables (2 & 3) revealed that antibacterial activity of leaf extracts in three solvents that the acetone leaf extract of Withania somnifera were the highest inhibition effect on the four species of pathogenic bacteria in the all concentration, the maximum inhibition zone recorded in 100 gm/ml were Pseudomonas aeruginosa 15 mm; klebsiella pneumonia 13 mm; the antibacterial activity on Gram positive bacteria were 13 mm for Staphylococcus aureus and 15 mm for Streptococcus pneumonia.

Methanol extract of leaf has strong inhibition activity against all multidrug resistance of pathogenic isolates, the range of their effect for different concentration about (8 -13 mm), the highest effect in 100 mg/ml concentration on (Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus aureus, and Streptococcus pneumonia) were the inhibition zone 12, 13, 11, 12 mm respectively.

The third types leaf extract were used in current study water extract showed the weak antibacterial activity on bacteria the lower inhibition zone observed in all concentration and species of bacteria isolate these
use in our study the diameter of growth inhibition zone on four pathogenic isolates by well diffusion method reach at highest concentration 100 gm / ml were 4 - 5 mm.

**Table (2) :** Anti bacterial effect of *Withania somnifera* leaves extract on Gram negative multidrug resistant isolates.

<table>
<thead>
<tr>
<th>Concentration mg / ml</th>
<th>Extract</th>
<th><em>Pseudomonas aeruginosa</em></th>
<th><em>Klebsiella pneumonia</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Inhibition zone / mm</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Acetone</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>50</td>
<td>Acetone</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>75</td>
<td>Acetone</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>100</td>
<td>Acetone</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

**Table (3) :** Anti bacterial effect of *Withania somnifera* leaves extract on Gram positive multidrug resistant isolates.

<table>
<thead>
<tr>
<th>Concentration mg / ml</th>
<th>Extract</th>
<th><em>Staphylococcus aureus</em></th>
<th><em>Streptococcus pneumonia</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Inhibition zone / mm</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Acetone</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>50</td>
<td>Acetone</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>75</td>
<td>Acetone</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>100</td>
<td>Acetone</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>
The result of the study agreement with the finding of previous finding that showed acetone extract of leaf has high a potent effect against pathogenic bacteria (20)(21). The high potential antibacterial of acetone and methanol leaf extract of *Withania somnifera* due to leaves components well extracted in polar solvent more than non polar solvent (22). The result of water extract became agreement with the some studies which showed that water extracts inhibited the growth Gram negative isolate due to withanolides compounds can by well extracted in water (23)(24) obtain similar result they showed the strong ability of crude extract of *Withania somnifera* to inhibition the growth five species of clinical bacterial isolates at 100 mg / ml concentration.

The strong inhibition effects on the growth of bacteria isolates of *Withania somnifera* extracts due to the active biochemical components as withanolides (major constituents of leaves), Amino acids, glycowithanolide, Alkaloids, flavonids and other compounds with the high effect to different diseases of extraction and purification(25).

**Conclusion**

Current investigation of antibacterial leaf extract of *Withania somnifera* showed that acetone and methanol extract exhibit strong activity against the four species of multidrug resistance pathogenic isolates, since we recommended to future studies to identify the chemical compounds to increase the drug activity and reduce the toxicity, to use the plant as antibacterial drugs for treating the disease specially for multidrug resistance isolates.

**Conflict of Interest:** non

**Source of Fundings:** self findings.

**Ethical Clearance:** non

**References**

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13- Jadhav, S., Hussain, A., Devi, S., Kumar, A., Parveen, S., Gandham, N., ... and Ahmed, N. Virulence characteristics and genetic affinities of multiple drug resistant uropathogenic Escherichia coli from a semi urban locality in India. PLoS
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The Diagnostic Role of Anti-Human Salivary Gland Protein-1 (anti-SP1) in the Early Detection of Primary Sjogren’s Syndrome in Some Iraqi Patients

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Abstract

Background: Primary Sjogren’s syndrome (pSS) is a chronic disorder described by its immune devastation to the salivary and lacrimal glands. The detection of pSS is complicated especially at the early stage. Thus, the emergence of novel markers preceding the conventional diagnostics for early detection of pSS before its aggravation is required. Objective: This study purposed to reveal the possible use of anti-SP1 as a beneficial indicator for early revelation of pSS in some Iraqi patients. Methods: Eighty-seven Iraqi patients (42 and 45 patients with newly diagnosed pSS and idiopathic sicca symptoms, respectively) enrolled in this study between February 2018 to July 2019. The diagnosis of pSS reliant on AECG and ACR criteria. All patients were assessed for RF, ANA, anti-Ro/SSA, and anti-SP1 by ELISA. Results: A greater prevalence of anti-SP1 was shown in pSS patients than control patients. Anti-SP1-positive pSS patients revealed significant shorter sicca duration with lowered Schirmer’s test and USFR, and predominant ANA and anti-Ro. Anti-SP1 evinced positive correlations with disease duration, ANA, and anti-Ro, and negative correlations with Schirmer’s test and USFR. Higher sensitivity (92.9%) and specificity (95.6%) of anti-SP1 in pSS patients than other diagnostic parameters. Conclusion: Despite small sample size, anti-SP1 still be a dependable marker for diagnosis of pSS at the early stage and discrimination of pSS patients than those with idiopathic sicca symptoms.

Keywords: primary Sjogren’s syndrome, anti-salivary gland protein-1 (anti-SP1), sicca symptoms

Introduction

Primary Sjögren’s syndrome (pSS) is a chronic autoimmune disorder that distinguish oneself by lymphocytic recruitment and devastation of the lacrimal and salivary glands with autoantibodies production, leading to sicca symptoms (eyes and mouth dryness) [1]. However, when the disease occurs alongside other autoimmune disorders like systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and systemic sclerosis, it is called secondary SS (sSS) [2].

The identification of pSS is complicated due to the lack of biomarkers with high efficiency and precision to prophesied the disease at the preliminary phase [3]. In addition, another diagnostic obstacle is that the sicca symptoms with limb pain and fatigue are very common in the people and may be accompanied with other pain syndromes [4].

Alternatively, the American-European Consensus Group (AECG) and American College of Rheumatology (ACR) created criteria to recognize pSS dependent on the utilization of clinical manifestations, measurements of sicca symptoms, histopathology of salivary/lacrimal glands, and autoantibodies production [5]. Several autoantibodies presently offer assistance to the identification of pSS include antinuclear autoantibody (ANA), rheumatoid factor (RF), anti-Ro/SSA, and anti-La/SSB whom may emerge with the disease’s exacerbation [6].

Consequently, alternate, non-invasive and dependable markers with high sensitivity and specificity are required to improve and simplify the early diagnostic process of pSS. Hence, novel biomarkers like anti-salivary gland protein-1 (SP1) were studied in mice and in patients with SS, which established to be manifested earlier in the disease phase [7].
The apparition of SP1 was originally in mice and Shen et al., five years later, evidenced identical proteins appeared in human salivary glands and the more significantly antibodies to SP1 were existent in pSS patients [8]. Besides, these autoantibodies were observed to be infrequently detected in healthy persons or patients with other autoimmune diseases [3].

The occurrence of anti-SP1, in several studies, has been detected in the early phase of pSS preceding anti-Ro/-La [9]. Notwithstanding, Theander and her colleagues study proclaimed anti-Ro/La might manifest a long time preceding the exhibition of pSS clinical features in particular patients [10]. Whilst the timing of anti-SP1 autoantibodies emergence in pSS patients is controversial, this study aims to disclose the bearable utilization of anti-SP1 as susceptible indicator for early identification of pSS.

**Materials and Method**

Patients: A cross-sectional study of 87 Iraqi patients (42 and 45 patients with newly diagnosed pSS and persistent idiopathic sicca symptoms (iSS), respectively) attended the Department of Rheumatology/Baghdad Teaching Hospital during the period from February 2018 to July 2019. The detection of pSS was dependent on the AECG and ACR classification criteria [5]. Patients that unachieved these criteria were classified as iSS. Patients with sSS or other rheumatic diseases, hepatitis viruses, acquired immuno-deficiency syndrome, IgG4-related disease, head/neck radiation, graft versus host disease, amyloidosis, and medications that may affect salivary/lacrimal gland function were excluded from the study.

**Result**

Significant differences amidst pSS patients and those with iSS regarding sicca duration, Schirmer’s test, USFR, ESR, and CRP were illustrated in Table (1). In contrary, non-significant differences were noted between the study groups for age and gender. However, RF, ANA, anti-Ro/SSA, and anti-SP1 were significantly prevalent in pSS patients.

**Table (1): Demographic distribution of the study groups.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>pSS (n=42)</th>
<th>iSS (n=45)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), mean±SD</td>
<td>47.0±10.2</td>
<td>50.1±10.6</td>
<td>0.180</td>
</tr>
<tr>
<td>Gender, male: female</td>
<td>11:31</td>
<td>13:29</td>
<td>0.286</td>
</tr>
<tr>
<td>Sicca duration (years), mean±SD</td>
<td>2.7±0.5</td>
<td>3.2±0.8</td>
<td>0.011</td>
</tr>
<tr>
<td>Schirmer’s test (mm/5 min), mean±SD</td>
<td>4.6±0.5</td>
<td>5.3±0.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>USFR (ml/15 min), mean±SD</td>
<td>0.09±0.03</td>
<td>0.13±0.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ESR (mm/hr), mean±SD</td>
<td>25.8±6.1</td>
<td>23.7±6.0</td>
<td>0.025</td>
</tr>
</tbody>
</table>
Table (2) showed significant differences among positive- and negative- anti-SP1 patients with pSS for sicca duration, Schirmer’s test, USFR, CRP, ANA and anti-Ro/SSA. Whilst, insignificant variances observed amidst those patients with pSS regarding age, gender, ESR, and RF.

A significant positive correlation of sicca duration, CRP, ESR, ANA, and anti-Ro/SSA, additionally, negative correlation of Schirmer’s test and USFR with anti-SP1 manifested in pSS patients. In contrast, non-significant correlation between study groups were noticed for age, gender, and RF with anti-SP1 (data viewed in Table 3).
Table (3): Correlation of anti-SP1 with other parameters among patients with pSS and iSS.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>pSS</th>
<th>iSS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>φ</td>
<td>P-value</td>
</tr>
<tr>
<td>Age</td>
<td>0.227*</td>
<td>0.540</td>
</tr>
<tr>
<td>Gender</td>
<td>0.255</td>
<td>0.098</td>
</tr>
<tr>
<td>Sicca duration</td>
<td>0.336</td>
<td>0.029</td>
</tr>
<tr>
<td>Schirmer’s test</td>
<td>-0.806</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>USFR</td>
<td>-0.469</td>
<td>0.002</td>
</tr>
<tr>
<td>CRP</td>
<td>0.305</td>
<td>0.048</td>
</tr>
<tr>
<td>ESR</td>
<td>0.439</td>
<td>0.004</td>
</tr>
<tr>
<td>RF</td>
<td>0.277</td>
<td>0.072</td>
</tr>
<tr>
<td>ANA</td>
<td>0.354</td>
<td>0.022</td>
</tr>
<tr>
<td>Anti-Ro/SSA</td>
<td>0.641</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Cramer’s V coefficient. φ (phi correlation coefficient)

Table (4) represented the diagnostic performance and accuracy of the studied parameters. The findings evidenced a higher sensitivity (92.9%), specificity (95.6%), PPV (95.1%), NPV (93.5%), PLR (20.9) and NLR (0.1) of anti-SP1 in pSS patients contrasting other diagnostic parameters.

Table (4): The diagnostic performance of the study parameters among pSS patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>PLR</th>
<th>NLR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schirmer’s test</td>
<td>92.0%</td>
<td>17.8%</td>
<td>51.3%</td>
<td>72.7%</td>
<td>1.1</td>
<td>0.4</td>
</tr>
<tr>
<td>USFR</td>
<td>88.1%</td>
<td>22.2%</td>
<td>51.4%</td>
<td>66.7%</td>
<td>1.1</td>
<td>0.5</td>
</tr>
<tr>
<td>RF</td>
<td>50.0%</td>
<td>62.2%</td>
<td>55.3%</td>
<td>57.1%</td>
<td>1.3</td>
<td>0.8</td>
</tr>
<tr>
<td>ANA</td>
<td>62.0%</td>
<td>60.0%</td>
<td>59.1%</td>
<td>62.8%</td>
<td>1.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Anti-Ro/SSA</td>
<td>71.4%</td>
<td>91.1%</td>
<td>88.2%</td>
<td>77.4%</td>
<td>8.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Anti-SP1</td>
<td>92.9%</td>
<td>95.6%</td>
<td>95.1%</td>
<td>93.5%</td>
<td>20.9</td>
<td>0.1</td>
</tr>
</tbody>
</table>

P (positive), N (negative), PV (predictive value) LR (likelihood ratio).
Discussion

The most debatable things is the time of autoantibodies appearance that may precede the clinical onset in pSS patients. The current study revealed significant elevation in patients with newly diagnosed pSS and those with persistent iSS for diagnostic parameters (Schirmer’s test, USFR, RF, ANA, anti-Ro, and anti-SP1). These results were consistent with findings by Lee et al., which showed a raised predominance of these diagnostic parameters in pSS patients comparing those iSS ones [13], suggesting the superior inflammatory pathways in patients with pSS than with sustained iSS. Nevertheless, the marker(s) that undoubtedly confirm suspected patients with pSS was needed, so significant higher prevalence of positive anti-SP1 was demonstrated in pSS than iSS patients comparing other diagnostic markers. Besides, positive anti-SP1 patients with pSS having elevated CRP and prevalent RF, ANA, and anti-Ro conflicting those with negative anti-SP1. Moreover, sicca duration, Schirmer’s test, and USRF were noticed markedly minimized in pSS patients with positive anti-SP1, suggesting the appearance of anti-SP1 in pSS patients with shorter duration and acute-phase. A study by Everett et al. was in harmony with our findings, they found that anti-SP1 was disclosed in pSS patients with ≤2 years duration and abnormal Schirmer’s test, and anti-Ro/La antibodies might not be detected before two-years of disease onset [14].

Referring to the possible replacement of anti-Ro/La with anti-SP1 in the early detection of pSS, Vishwanath et al. study reported iSS patients with negative Ro/La antibodies exhibited positive anti-SP1, hence, tissue-specific autoantibodies (e.g. anti-SP1) are reliable markers for early identification of pSS [15]. In contrary, the findings by Suresh et al. were discrepancy with our results as they found (based on their study design) that anti-Ro/La, in contrast to anti-SP1, might be linked with the disease’s acuteness and exhibited higher sensitivity in the discrimination between normal and diseased individuals [16].

Several explanations may clarify the earlier emergence of anti-SP1, contrasting anti-Ro/La, during the early stage of pSS. Among them the early immunogenic inflammatory processes that may occur initially in the salivary and lacrimal glands destroying the epithelial cells with activation of B cells via the recruitment of follicular T-helper cells [17]. Besides, patients with pSS may develop hyperglobulinemia that leads to the formation and potentially deposition of immune-complexes in the secretory glands causing irreversible damage with the expression of epithelial cells contents and stimulation of chronic immune responses [18].

Another explanation, both Ro and La are parts of extractable nuclear antigens that existed in many cell types, therefore anti-Ro/La can be found in several autoimmune disorders resulting in decreased their specificity to pSS particularly at the early phase [19]. In return, the tissue specificity of SP1 (salivary gland secretory protein) making anti-SP1 potentially tissue-specific antibodies that may correlate with the early diagnosis of pSS due to the initial inflammatory devastation of the salivary and lacrimal glands [20].

The study findings displayed that anti-SP1 antibodies were correlated positively with the sicca duration, the inflammatory markers, ANA, and anti-Ro, as well as, correlated negatively with the clinical signs’ measurements in pSS patients. Therefore, this relationship of anti-SP1 with the shorter disease duration, and the inflammatory as well as immunological markers indicating the involvement of immunoinflammatory reactions in the salivary glands with early emergence of these autoantibodies during the disease onset. Phung et al. [21] referred that anti-SP1 was more specific than anti-Ro/La for early identification of SS since SP1 was located particularly in the salivary glands contrasting Ro/La that existed in any nucleated cells. Likewise, the anti-SP1 was rarely detected in healthy persons (<5%), and the patients suffering early SS have >60% of novel autoantibodies (e.g. anti-SP1) and 20-30% of anti-Ro/La, whereas in patients with late SS these proportions were inverted. Karakus et al. declared that anti-SP1 discovered in >20% of SS patients contrasting 13% of iSS patients, also anti-SP1 was the only autoantibody correlated significantly in those with ≤5 mm of Schirmer’s test [22].

The diagnostic performance of the studied parameters elucidated excellent performance of anti-SP1 with higher sensitivity (92.9%) and specificity (95.6%) in the pSS detection comparing other parameters, suggesting the possible utilization of anti-SP1 antibody for early detection of pSS due to its higher specificity to the salivary glands, and eventually more sensitive to the pSS especially at the early stage. Jin et al. stated that anti-SP1 elevated obviously in patients suffering early pSS with shorter disease duration and negative anti-Ro/La antibodies; indicating the effective use of these
autoantibodies as a diagnostic marker particularly during the early stage at which anti-Ro/La antibodies were negative[23]. In conclusion, notwithstanding small sample size, the current findings are still worthy to proclaim the beneficial use of anti-SP1 as a marker for pSS detection especially at the early phase and discrimination patients suffering pSS from those with iSS. Molecular studies with a larger cohort are required to estimate the time of SP1 expression in human salivary gland for possible use of anti-SP1 as a predictor of pSS.

Acknowledgment: Our thanks for rheumatologist Dr. Aimen Abbas and the staff of the laboratory division in Baghdad Teaching Hospital for their assistance.

Conflict of Interest: non

Source of Findings: Self findings.

Ethical Clearance: Non

References


Effect of Sunlight and Vitamin D3 on some Men Fertility Parameters

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Abstract

The current study aims to clarify the effect of the exposure to sunlight on the vitamin D level and the effect of this on characteristics of seminal fluid and some hormones in infertile men. The study included 45 men (aged between 18-44 years) who suffer from infertility. These men attend the Infertility Treatment Centre at Kut Hospital, Wasit, Iraq. Infertile men were exposed to the sunlight without using sunscreen for ten minutes at daily intervals over 3 months. Seminal fluid parameters, as well as the level of Vitamin D3, Follicle-stimulating hormone (FSH), Luteinizing hormone (LH) and Testosterone, were measured before and after exposure to sunlight. The study showed that there was a significant increase in count, activity and normal morphology of seminal fluid as well as elevation in LH, FSH, testosterone and vitamin D3 level in blood after the exposure to sunlight.

Keywords: Sunlight, Seminal Fluid, Vitamin D3, LH, FSH, Testosterone.

Introduction

Sunshine contains ultraviolet (UV) rays, which are three types: type A (320 nm - 400 nm), known as UVA, which causes skin damage and the appearance of signs of aging, type B (290 nm - 320 nm) and symbolized by the (UVB) and type C (100 nm - 290 nm) known as UVC which is absorbed mostly by the ozone layer at the upper atmosphere and does not reach to the earth ¹. UVB gives the skin the energy needed to make vitamin D3 when 7-dihydroxy cholesterol reacts with ultraviolet (type B) at wavelengths between 270 and 300 nm, and peak formation is between 295 and 297 nm. ². These wavelengths are in sunlight when the UV index is greater than three ³. Vitamin D manufacture in the human skin following sun exposure is depend on the location of the sun in the sky and the zenith angle ⁴. Zenith angle depends on the latitude, season, weather conditions, and time of the day ⁵. even though the sun is closer to the earth in winter, it enters a more slanted angle (azimuth angle) and more UV radiation is absorbed by the ozone layer and passing in more distance which leading to fewer UV rays reaching to earth ⁶. The latitude above 37 ° affects the angle of the sun’s peak from November to February, where there is a marked decrease in the amount of UV radiation reaching to the earth surface. Therefore, very little vitamin D3 is produced in the skin during the winter, while the latitude less than 37 degrees and closer to the equator, the production of vitamin D3 in skin is higher throughout the year ⁷. In the case of the first morning or late in the day, the azimuth angle is highly tilted and the production of vitamin D3 is very low in the skin even in the summer and exposure to the sun is need between 1000 and 1500 hours, as a result, the optimal times for vitamin D manufacture in human skin is in the summer among the hours of 10 AM and 3 PM ⁸. A 15-minute exposure to sunlight on the face, arms, and legs without sunscreen is enough to produce 1500-3000 vitamin D units. This is enough for one to three days ⁹.

Generally, exposure to sunlight for 15-20 minutes three times a week is safe, does not cause skin damage, and enough to provide healthy amounts of vitamin D to the body ¹⁰. Dark-skinned people have increased melamine, which absorbs UV rays more effectively, and therefore need longer exposure to the sun to get the similar amount of vitamin D3 when compared with people with white skin ¹¹. It should be noted that the production of vitamin D3 by sunlight has a comparative advantage, such as fish oil, where the production of vitamin D through the sun does not reach the excess rates or harmful. As there is a process of balance performed by the skin automatically, and the excess of vitamin is eliminated ¹². Vitamin D that produces by skin or comes from the diet is physically inactive and need to add hydroxyl group in the liver.
by aidthe 25-hydroxylase enzyme to 25(OH)D. Also, vitamin 25(OH)D needto additional hydroxyl group in the kidney byenzyme 25(OH)D-1-OH-ase to produce 1,25(OH)2D the physically active of this vitamin. Vitamin D is one of the most powerful vitamins necessary for bone health because it helps absorb important calcium for bone and dental health and plays a vital role in building muscle mass, strengthening the immune system and nerve signals. According to some research, vitamin D affects the health of internal cells. The lack of vitamin D in men leads to what is known as erectile dysfunction problems due to weak blood flow in the male genitalia, so men are not able to have an erection, which results in erectile dysfunction. Another study showed that sexual desire is linked to the level testosterone is associated with vitamin D. The elevated the level of vitamin D, the elevated the testosterone. When the vitamin decreases, the sexual ability of men is diminished. This is mostly in the winter because of the lack of the sun to supply vitamin D, which raises the level of testosterone.

Several factors contribute to the process of production and activation of sperm and sexual activity for men, including vitamin D, which shows that the presence of the receptor on the head and the med body of sperm. Vitamin D is essential in the absorption and metabolism of calcium where it is vital for spermatogenesis and sperm activity.

**Materials and Method**

The study was conducted on 45 infertile males between the ages of 18-45 years and who attended the Infertility Treatment Center at Kut Hospital, Wasit, Iraq. The information required for the study was collected from the subjects including age and duration of the marriage. Also, we looked at the previous surgery, stimulant therapies for the reproductive system during the past 6 months, and chronic diseases with excluded smoking persons.

Semen samples were collected in a clean screw plastic tube by masturbation method from subjects after a period of abstinence for 3-5 days and placed in the incubator at 37 °C to allow them to have normal liquefaction. After the liquefaction occurred, the semen was examined for sperm count, movement, availability and sperm morphology. The blood samples were collected in sterile plastic tubes and the serum separated by centrifugation at 3000 rpm for estimating of hormones and vitamin D3 before exposure to sunlight and sent to the laboratory immediately for analysis. Participants then exposed to direct sunlight for continuous 10 minutes daily during 10.0 am and 3.0 pm o’clock without using any sunscreen for three months from 1-3 to 1-6-2019, samples of semen and blood collected after the end of the sunlight exposure and sent to laboratory for analysis. Seminal fluid concentration and motility estimated by using a Microcells counting chamber methodand sperm morphology estimated by use Diff-Quick kit according to World Health Organization criteria. 25-hydroxyvitamin D (vitamin D3), total testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH), were estimated by using Electrochemiluminescence-Immunoassay (ECLIA) kits.

**Statistical Analysis**

Statistical analysis was completed with use the student T-test. The results were expressed as a mean and standard-deviation (SD). The P values of ≤ 0.01 were expressed as significant values.

**Finding**

Study showed there were significant increase in serum vitamin D3 (42±2.62) in men after exposed to sunshine as compare with before exposure (28±1.59) (table 1, figure 1), significant increase in serum LH (7.13 ± 1.37) in men after exposed to sunshine as compare with before exposure (5.06 ± 2.77), significant increase in serum FSH (6.24 ± 2.58) in men after exposed to sunshine as compare with before exposed (4.87 ± 1.75) and significant increase in serum testosterone (12.84 ± 2.77) in men after exposed to sunshine as compare with before exposed (9.23 ± 3.82) (table 1, figure 2). In addition, the study showed a significant increase in sperm count (42.23 ± 29.8) in men after exposed to sunshine as compare with before exposed (28.72 ± 23.48), significant increase in sperm active motility (29.07 ± 20.2) in men after exposed compared with before exposed (12.93 ± 11.92) significant increase in normal sperm viability (45.26 ± 27.4) in men after exposed to sunshine as compare with before exposed (28.33 ± 18.6) and showed a significant increase in normal sperm morphology (58± 11.54) in men after exposed to sunshine as compare with before exposed (39.5 ± 16.49) (Table-2, Figure-3).
Table (1):- The mean and standard-deviation of serum vitamin D3, LH, FSH and testosterone hormones before and after exposure to sunshine

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before sunlight exposure</th>
<th>After sunlight exposure</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D3(ng/ml)</td>
<td>28 ± 1.59</td>
<td>42 ± 2.62</td>
<td>P&lt;0.001*</td>
</tr>
<tr>
<td>Serum LH (mlU/ml)</td>
<td>5.06 ± 2.77</td>
<td>7.13 ± 1.37</td>
<td>P&lt;0.001*</td>
</tr>
<tr>
<td>Serum FSH (mlU/ml)</td>
<td>4.87 ± 1.75</td>
<td>6.24 ± 2.58</td>
<td>P&lt;0.0001*</td>
</tr>
<tr>
<td>Serum testosterone (ng/ml)</td>
<td>9.23 ± 3.82</td>
<td>12.84 ± 2.77</td>
<td>P&lt;0.001*</td>
</tr>
</tbody>
</table>

P value ≤ 0.01 were expressed as significant values*

Figure (1):- Show Vitamin D level before and after exposure to sunlight.

Figure 2: Show the level of LH, FSH, and testosterone hormones before and after exposure to sunlight.
Table 2: Mean and standard-deviation of sperm parameters before and after exposure to sunshine

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before sunlight exposure</th>
<th>After sunlight exposure</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm count (Million/ml)</td>
<td>28.72 ± 23.48</td>
<td>42.23 ± 29.8</td>
<td>0.01*</td>
</tr>
<tr>
<td>Sperm active motility (%)</td>
<td>12.93 ± 11.92</td>
<td>29.07 ± 20.2</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Sperm viability (%)</td>
<td>28.33 ± 18.6</td>
<td>45.26 ± 27.4</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Normal Sperm morphology (%)</td>
<td>39.5 ± 16.49</td>
<td>58 ± 11.54</td>
<td>0.0001*</td>
</tr>
</tbody>
</table>

P value ≤ 0.01 were expressed as significant values*

Figure 3: Show count, motility, viability and morphology of sperm before and after exposure to sunlight

### Discussion

The present study showed that there is a significant increase in the level of vitamin D3 that formed after the skin is exposed to direct UVB sunshine 10 min daily for 3 months and this elevation has a direct effect on reproductive testosterone hormone and increases the level of testosterone and vitamin D, which in turn, causes increased release of LH and FSH hormone. Eventually, this leads to increase count, motility, viability, and morphology of sperm. Also, it increases the level of vitamin D3 when the skin exposed to direct sunlight.

This is conformity with many earlier studies shown the association between sunlight and vitamin D3 where skin exposure to the direct UVB sunlight 7-dehydrocholesterol in the skin changed to pre-vitamin D3 which is changed in the liver to vitamin D3. The study also showed that there is a noticeable increase in the level of reproductive hormones LH, FSH, testosterone after exposure to direct sunlight and this is due to the effect of the increase in the level of vitamin D3 and this is consistent with previous research.

Some studies have confirmed the relation between the presence of vitamin D receptors (VDR) on the body of sperm and sperm activity and any deficiency in the level of vitamin negatively affects the activity of sperm and also through the future, vitamin D regulates the level of calcium necessary for the reproductive system and sperm activity.
Conclusion

Sunlight causes increase vitamin D3 level by action of UVB ray this lead to increase in the level of the reproductive hormones testosterone, LH and FSH, increase this parameter improve fertility by increase seminal fluid total count, actively motile, viability and normal morphology.

Recommendation

Expose to sunlight at least 10 min daily from 10.0 am to 3.0 pm o’clock to improve the fertility of infertile men before taking any drug for this purpose.

Conflict of Interest: Non

Source of Findings: Self findings.

Ethical Clearance: Non

References


additional calcium to reduce the risk of hip fracture with vitamin D supplementation: evidence from a comparative metaanalysis of randomized controlled trials. J Clin Endocrinol Metab 2007; 92: 1415–23


Effect of Different Die Materials on the Marginal Fit of CAD/CAM crowns pre and pos -cementation

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¹Department of Prosthetic dental technologies ,College of Health and medical Technology middle Technical University, Baghdad, Iraq, ²Department of conservative dentistry, College of Dentistry, University Baghdad of Iraq

Abstract

The objective of this in vitro study was to evaluate the vertical marginal gap pre and post-cementation of IPS e.Max CAD crowns constructed using different die materials.

Materials and method: The thirty standard aluminum dies were fabricated to receive a full contour IPS e.Max CAD crowns. An impression with two-stage putty-wash impression with spacer technique was taken. The thirty impressions were distributed into three groups as follows; Group (A) Ten impressions were poured with type IV die stone, Group (B) Ten impressions were poured with type V die stone and Group (C) Ten impressions were poured with Exacto-form model resin die material. Each die was scanned and IPS e.Max CAD full contour crown was fabricated using Sirona CEREC in-Lab CAD/CAM System. Sixteen gap measurements were taken for each sample (Buccal, Mesial, Lingual and Distal), Pre and post-cementation process were measured using a digital microscope. The results of this study showed that there were statistically highly significant differences (P<0.01) between three different die materials groups of pre and post-cementation procedure. From the results of this study, it could be concluded that the Exacto-form provided the least vertical gap, when used IPS e.Max CAD full contour restorations. Die stone type V could be the next choice, which is preferred over to die stone type IV. Cementation step increased the marginal gap significantly.

Keywords: Die Materials, IPS e.max CAD, CAD/CAM , Pre and Post- Cementation Procedure, Marginal Fit.

Introduction

To obtain accurate and precise models with no distortion, it is important to acquire accurate impressions and to use stable and precise die materials. Die material is one of the factors affecting the accuracy of the replica[1] Type IV, type V gypsum (die stone) and polyurethane resin (Exatofrom) were widely used to pour the impressions. The properties of gypsum include high strength, low expansion easy to use, and allowing easy separation of the working cast from the impression. Polyurethane resin (Exatofrom) has been also used as die material for its accurate reproduction and maximum edge stability, extremely high fluidity of this resin allow pouring of impression with high precision and without the formation of bubbles[2].

Material and Method

A dentoform maxillary right first molar tooth (Dentoform, Nissin, Kyoto, Japan) was used in this study as an in vitro model and was duplicated to aluminum dies. The dentoform tooth then received a preparation with the following features: planar occlusal reduction of 1.5 mm, axial reduction of 1-1.5 mm, 0.8 mm circumferential chamfer finishing line and a 6° total convergence angle a modified dental surveyor. The prepared dentoform tooth was to fabricate 30 aluminum dies for each aluminum dieconventional impression using two-step putty/wash impression technique with spacer was taken using a specially designed special tray for this study. The impressions were randomly all located into three groups of 10 each according to the type of die material; Group A: die stone type IV (SHER AHRAD-ROCK ISO 6873, LOT78083LemfÖrde, Germany); Group B: die stone type V (schouten group dental N chang: 17.2595 Netherland); and Group C: Exatofrom resin die materials (Bredent,Senden, Germany LOT No.52000173). The die materials were mixed and the impression were poured with the die materials following the manufacturers instructions. After setting, the dies
were separated from their impressions and checked for any defect as air bubbles or imperfection.

In EosX Scanner (Sirona dental system, bensheim. Germany) was used to make ascans for each die model and MCX5 milling unite was used to millIPS e.max CAD crowns with 120µm spacer parameter. The marginal gap of the each sample was determined by seating the e-max CAD crown on its own aluminum die with the aid of a specimen holding device to provide a standard load of 5 Kg. Adigital microscope (Dino-Lite, Taiwan), was used at magnification of 230X connected to the computer to capture the images which were processed with Image J software to measure the marginal gap. Sixteen points were selected for measurements, 4 for each surface and the average was calculated for each specimen(3); the crowns were then cemented using Rely X U200 (3M ESPE, USA) self-adhesive resin cement with the aid of a surveyor under 5 Kg load(4). After cementation, the same points of measurements were re-examined again using the same procedure described previously to calculate the vertical marginal gap.

**Findings**

Table (1) shows the descriptive statistics which includes (mean and standard deviation values) of vertical marginal gaps pre-cementation. The results is evident that the lowest mean of vertical marginal gap values was scored by group C (55.073±6.299) (while the highest mean of vertical marginal gap values was belonged to group A (76.517±9.152). One-way ANOVA and LSD tests between the studied groups was a highly significant difference (P< 0.01).

The means and standard deviations of the marginal gap which were calculated for each group post-cementation are shown in (Table 2) and it showed that lowest mean value was recorded by group (C) which was 73.442±6.007 while the highest mean value recorded by group (A) which was equal to 94.0706±6.694. One-way ANOVA and LSD tests between the studied groups was a highly significant difference (P< 0.01).

Paired samples t-test was used to compare between the e.Max CAD crowns per-cementation and post-cementation , Table (3) and Figure(3) It is evident that the cement on increased the vertical marginal gap was statistically highly significant degree for all groups.

**Table (1) Mean distributions of the vertical marginal gaps for the three different die materials groups measured pre-cementation (in micrometer)**

<table>
<thead>
<tr>
<th>Studied groups</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>ANOVA Test (P-value)</th>
<th>LSD test (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (Die stone type IV)</td>
<td>10</td>
<td>76.517</td>
<td>9.152</td>
<td>P=0.00</td>
<td>P=0.00</td>
</tr>
<tr>
<td>Group B (Die stone type V)</td>
<td>10</td>
<td>65.310</td>
<td>3.433</td>
<td>High sign. (P&lt; 0.01),</td>
<td>High sign. (P&lt; 0.01).</td>
</tr>
<tr>
<td>Group C (Exakto-form)</td>
<td>10</td>
<td>55.072</td>
<td>6.299</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table (2) Mean distributions of the vertical marginal gaps for the three different die materials groups measured post-cementation (in micrometer)**

<table>
<thead>
<tr>
<th>Studied groups</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>ANOVA test (P-value)</th>
<th>LSD test (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (Die stone type IV)</td>
<td>10</td>
<td>94.071</td>
<td>6.694</td>
<td>P=0.00</td>
<td></td>
</tr>
<tr>
<td>Group B (Die stone type V)</td>
<td>10</td>
<td>86.965</td>
<td>11.067</td>
<td>High sign. (P&lt; 0.01),</td>
<td></td>
</tr>
<tr>
<td>Group C (Exakto-form)</td>
<td>10</td>
<td>73.442</td>
<td>6.007</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table (3): Paired Samples t-test between all groups’ pre- and post- cementation.

<table>
<thead>
<tr>
<th>Groups</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>A. pre Vs. A.post</td>
</tr>
<tr>
<td>Group B</td>
<td>B. pre VS B. Post</td>
</tr>
<tr>
<td>Group C</td>
<td>C. pre VS C. Post</td>
</tr>
</tbody>
</table>

Discussion

High marginal accuracy is considered to be major determining factor for successful clinical performance of a restoration[6].

The results of this study showed that mean value of vertical gap of the three different groups revealed that at group(C) (Exacto-form) produced statistically the least vertical marginal gap both pre- and post-cementation. The findings was in agreement with [6] who that the mean marginal gap was consistently larger for type IV stone dies than polyurethane resin dies. The superiority of Exacto-form regarding vertical marginal gap in this study could be attributed to:

Figure (1): mean values of the marginal gaps for three groups pre- and post-cementation (in micrometer).

Figure(2)Microscopical image of the marginal gap for group A, Band C at a magnification of 230X pre-cementation procedure, (A: Crown, B: Marginal gap, C: Aluminum die).

Figure(3)Microscopical image of the marginal gap for group A, Band C at magnification of 230X post-cementation procedure, (A: Crown, B: Marginal gap, C: Aluminum die).
A. **Dimensional accuracy:** Gypsum die materials have been reported to exhibit setting expansion of 0.01 to 0.1%. This minimal expansion has been said to compensate for the dimensional changes inherent in the fabrication process of an indirect restoration. The process of gypsum crystallization is an expansive growth of crystals from a core of crystallization. Based on the interlacing of dihydrate crystals, the growth of the core crystals can combine and block the growth of adjacent crystals. If this process is repeated by thousands of crystals during their growth, an external tension will be developed that leads to “expansion of the mass, and the product of the gypsum reaction is larger than its external volume but smaller in crystalline volume due to dimensional change of die after final set”\(^1\). On the other hand, polyurethane resin (Exacto-form) was showed lower mean values in the vertical gaps, during polymerization the materials presents low shrinkage (0.060%) this change however was compensated and controlled by increasing the silica filler particles of the mix to 60% resulted in lower polymerization shrinkage of 0.025% to improve the dimensional accuracy of polyurethane die lead to more accuracy fit of indirect full veneer crown comparison gypsum die materials\(^7\).

B. **Detail reproduction:** Polyurethane resin has ability to reproduce details of up 1-2µm contrary to gypsum material which cannot reproduce details smaller than 20µm due to its crystal structure. With gypsum dies air bubbles are often formed at the interface of the impression and gypsum cast because freshly mixed gypsum does not wet some elastomeric impression materials led to it do not reproduce surface detail resin die materials because the surface of the set gypsum is porous on a microscopic level thus; polyurethane resin “Exacto-form” showed the best performance to the fine details present in this gypsum die\(^8\).

The results of this study revealed that at group (A) produced statistically the highest vertical marginal gap than group (B) both pre- and post-cementation in this study could be attributed to.

A. **Dimensional accuracy:** Type 4 gypsum differs from type 5 in that type 4, die stone V contain small amounts of extra salts (Sodium chloride) for shortens the setting reaction due to increased more number of nuclei of crystallization per unit volume increases, the setting expansion of the gypsum mass increases of 0.3% of type V stones than the 0.1% setting expansion of type IV stones, the desirable in this technique because it compensates for the contraction of the framework metals especially base metals\(^9,10,11\). On the other hand, setting expansion of the gypsum mass decreases it causes deformation of the prepared tooth was removed from impression might be another possible source for such inaccuracy\(^12\).

B. **Detail reproduction:** Die stone type V has ability to reproduce details than the die stone type IV because it had on Sodium citrate is a dependable retarder. Borax, Na2B4O7, is both a retarder and accelerator. A mixture of calcium oxide (0.1%) and gum arabic (1%) resulting in improved properties, compressive strength, hardness, abrasion resistant and reproduction details than the die stone type V, all properties with effected direct impact on fitted of final restoration\(^7,13,14\).

2. **Effect of cementation process**

The results showed that after cementation, the vertical marginal gap increased significantly. The cement layer may increase the marginal gap uncontrollably and unequally depending on the cement viscosity. One study revealed that the marginal gap increased by 18 to 22 µm when the crown was luted with cement. The high viscosity in self-adhesive cement resin create the problem of escape of excess cement and hydraulic pressure that is going to push the cement upward, this will result in great amount of luting cement to be accumulated on the occlusal surface of the prepared tooth, probably caused interfere with proper seating of crown restoration and prevents the crown from complete seating post-cementation process\(^15,16\). Vertical marginal gap values were increased significantly post-cementation; this is in total agreement with other previous studies: \(^5,17,18,19,20,21\).

**Conclusions**

Exacto-form resin showed better vertical and horizontal fitness than both die stone type IV and die stone type V. Cementation process significantly increased the vertical marginal gap values for all groups regardless the type of die materials but still within the clinically acceptable (< 120 µm).

**Conflict of Interest:** non

**Source of Findings:** self

**Ethical Clearance:** This research was carried out with the patients.
References


Evaluation Inhibin B, FSH, and LH in Male with Thalassemia

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¹Medical Laboratory Technology Department, Bilad Al-Rafidain University College.²Medical Laboratory Technology Department, Erbil Polytechnic University.³Faculty of Pharmacy, Bilad Al-Rafidain University College.

Abstract

Background : - Hypogonadism is the most frequently reported endocrine problem, affecting 70–80% of patients with thalassemia. Aim of study: - The current study is aimed to found correlation among inhibin-B, FSH, LH and testosterone in male with thalassemia. Material and Subject: The study carried out 40 healthy volunteer male, age range between (18-30) years, and enrolled 40 patients with thalassemia major age range between 18-30 years. The concentrations serum inhibin-B was estimated by two-side enzyme-linked immunosorbent assays (ELISA), While FSH, and LH estimated by using Cobas e analyzers. Results: LH reduce significantly in thalassemia patients when compared with healthy individuals, while FSH and testosterone decrease significantly in thalassemia patients when compared with healthy. Inhibin B was reduced significantly in thalassemia patients when compared with healthy individuals. Conclusion: Infertility common in thalassemia patients, that correlation with decrease inhibin B. Key Words: - Inhibin B, FSH, LH and thalassemia

Introduction

Beta-thalassemia is a genetic blood disorder characterized by absent or β globin chain synthesis, resulting in decrease hemoglobin in red blood cells, reduced RBCs production and anemia[1].

The direct effect is an imbalance of the α and β globin chain synthesis that results in anemia from ineffective erythropoiesis and hemolysis[2].

The severity and prevalence of hypogonadism in thalassemia major differs among studies, dependent on the genotype of thalassemia and age group studied[3][4][5][6][7]. Dysfunction of the sexual and infertility due to hypogonadism are well-recognized disorders of the hypothalamic pituitary gonadal axis[8].

Hypogonadism is the most frequently reported endocrine problem, affecting 70–80% of patients with thalassemia. Luteinizing hormone (LH) and Follicle-stimulating hormone (FSH) are involved in the regulation and activation of the reproductive axis. Hormones produced via the gonad control LH and FSH synthesis and excretion in a feedback loop[9]. Inhibin-B exhibit a physiological role in the feedback regulate of FSH secretion, and reflects FSH-stimulated Sertoli cell to be the function[10]. Testicular functions can be evaluated via the basal hormonal FSH and LH[3].

Inhibin’s are presently predictable as paracrine testicular controllers and have many paracrine effects representing a promising marker for infertility in male[11].

Aim of study: - The current study is aimed to found correlation among inhibin-B, FSH, LH and testosterone in male with thalassemia.

Patients and Method

The study carried out 40 healthy volunteer male, age range between 18-30 years, and enrolled 40 patients with thalassemia major age range between 18-30 years. All patients received blood transfusion every 4-5 weeks.

The concentrations inhibin-B was estimated via two-side enzyme-linked immunosorbent assays (ELISA), While FSH, and LH estimated by using Cobas e analyzers.

All data are stated as mean ± stander error. The comparisons among groups were completed via t test.

The relationship between inhibin B, testosterone, FSH, and LH were determined using a pearson-correlation coefficient. P less 0.05 was measured significant. The SPSS software for windows, version 20 were achieved to all analyses.
Findings

Table 1 illustrated fertility parameters and inhibin B in male with thalassemia, the result showed LH within normal range (1.7-8.6mlU/ml) in thalassemia patients (3.37±0.62 mlU/ml) but reduce significantly when compared with healthy individuals (8.60±0.26 mlU/ml) p<0.000, while FSH and testosterone decrease than the normal range (0.5-4.3 ml/mL, and 3-10 ng/mL) respectively in thalassemia patients (3.07±0.31mlU/mL, and 2.20±0.28ng/mL) respectively, as well as decrease significantly when compared with healthy individuals (6.52±0.34mlU/ml, and 7.08±0.29ng/mL) respectively p<0.000. Inhibin B was within the normal range (25-325pg/mL) in thalassemia patients (45.61±4.69pg/mL), and also reduce significantly when compared with healthy individuals (102.14±3.10/mL) respectively p<0.000.

Table 1: Inhibin B, Testosterone, FSH, and LH

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal range</th>
<th>Healthy Individuals (N=30) M±SE</th>
<th>Thalassemia Patients (N=60) M±SE</th>
<th>T-test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibin B</td>
<td>25-325pg/mL</td>
<td>102.14±3.10</td>
<td>45.61±4.69</td>
<td>&lt;0.000</td>
<td></td>
</tr>
<tr>
<td>Testosterone</td>
<td>3-10ng/mL</td>
<td>7.08±0.29</td>
<td>2.20±0.28</td>
<td>&lt;0.000</td>
<td></td>
</tr>
<tr>
<td>FSH</td>
<td>0.5-4.3mlU/mL</td>
<td>6.52±0.34</td>
<td>3.07±0.31</td>
<td>&lt;0.000</td>
<td></td>
</tr>
<tr>
<td>LH</td>
<td>1.7-8.6 mlU/mL</td>
<td>8.60±0.26</td>
<td>3.37±0.62</td>
<td>&lt;0.000</td>
<td></td>
</tr>
</tbody>
</table>

M Mean, SE Stander error, LH Luteinizing Hormone, FSH Follicular Stimulating Hormone

Table illustrated relationship among the changes in inhibin B with testosterone, FSH and LH, The mean inhibin-B was significantly positive correlation with mean testosterone, FSH and LH (r=0.497, p=0.001, r=0.333, p=0.036), and (r=0.447, p=0.004) respectively in thalassemia patients Table (2).

Table: 2 Correlation among Inhibin B, Testosterone, FSH, and LH

<table>
<thead>
<tr>
<th>Inhibin B</th>
<th>Parameters</th>
<th>Pearson Correlation</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Testosterone</td>
<td>0.497</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>FSH</td>
<td>0.333</td>
<td>0.036</td>
</tr>
<tr>
<td></td>
<td>LH</td>
<td>0.447</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Discussion

Physiological inhibin-B production via the adult testis needs a normal population of Sertoli cells, Spermatogenesis and FSH stimulation, to be existing and it displays an significant role in feedback hormone regulation between the gonads and the pituitary gland\[12\][11]. Current results show a significant positive correlation among inhibin-B, FSH and LH in male with thalassemia. Bergada et al. 2002 report a significant positive correlation of men inhibin-B with men LH\[13\]. These results then funding the inhibin-B has a role in the physiological regulation of LH and FSH excretion in male and inhibin-B is the testicular feedback signal for FSH\[14\]. Testosterone and inhibin-B
and originate from different forms of cells in the testis [15]. In our results we found positive correlation among inhibin-B and testosterone concentrations. Inhibin excretion from Sertoli cells is controlled via interaction with germ cells and expression of α- and βB-subunit mRNA is maximal at stages of spermatogenesis which are greatly sensitive to FSH [16][17][18]. The mean values of inhibin B, LH, FSH and testosterone, in this study, there was highly significant decrease in thalassemia patients when compared with healthy individuals. An earlier study displayed that histological investigation of the testicular tissue in patients with thalassemia proved variable degrees of fibrosis in testicular interstitial with small deeply pigmented undifferentiated seminiferous tubules, hyalinized, and an absence of Leydig cells[19]. Because of developments in the current medical care systems, males with β-thalassemia major who receive life-long blood transfusions now live longer. Therefore, the fertility and reproductive endocrinology requirements for such men are becoming more significant. The results of this study showed a significant decrease in the level of pituitary and testicular hormones, and this may afford additional strong evidence to suggest that iron overload at high risk of being related with reduced function of gonad[20].

Serum Inhibin concentration have been stated useful to evaluate testicular function in many conditions [21]. In this study, they were lower than the healthy controls. This suggested that Sertoli cell function is abnormal in thalassemia patients and regular blood transfusions[22].

Serum testosterone levels, FSH and LH in patients with major were different significantly when compared with healthy controls suggested that the hypothalamic–pituitary–gonadal axis is defect. In addition, serum testosterone levels in thalassemia patients were lesser than in controls. This suggested that testicular function is weakened[23]. Other causes of these abnormalities may be related to the catabolic iron catalyzes the production of free radicals, resulting in oxidative stress in lipid membranes, lysosomes, mitochondria, DNA, proteins. This is maybe induced oxidative stress and direct effect in many organs, like, pituitary gland, hypothalamus, and female reproductive organs, other organs indirectly effected such as, pancreas and liver, and this will be contributing to the impaired the processes of metabolism of serum antioxidants and hormones [24]. Oxidative stress modulates the age-related decline in infertility in patients suffer from β-thalassemia [25].

Conclusion

Infertility common in thalassemia patients, that correlation with decrease inhibin B.

Conflict of Interest: Non

Source of Findings: Self findings.

Ethical Clearance: Non

References


[25] Al-Gubory, K.H., Garrel, C., Faure, P. and Sugino, N. Roles of antioxidant enzymes in corpus luteum rescue from reactive oxygen species-induced oxidative stress, Reproductive Biomedicine Online, 2012; vol. 25, no. 6, pp. 551–560,

Anti-phosphoryl Choline and hs - CRP in Serum of Atherosclerosis Cardiovascular Patient with Chronic Periodontitis

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1College of Dentistry, University of Baghdad/Iraq

Abstract

Background: Periodontitis and Atherosclerosis Cardiovascular disease are chronic inflammatory diseases which are highly prevalent. Over the last two decades, the amount of evidence corroborating an association between dental plaque bacteria and coronary diseases that develop as a result of atherosclerosis has increased.

The aim of study: was to evaluate the periodontal health status in study groups (Atherosclerotic cardiovascular disease patients with chronic periodontitis (ATH+CP) and patients having chronic periodontitis (CP)), to investigate the serum level of antiphosphorylcholine antibody-IgG(Anti-PC IgG), and the systemic level of inflammatory marker of cardiovascular diseases like high sensitivity C-reactive protein (hs-CRP) in chronic periodontitis with and without atherosclerosis cardiovascular disease as well as to examine the relationships between these mediators and clinical periodontal parameters.

Material and Methods: Blood samples were collected from sixty patients (30 CP patients and 30 CP+ATH patients) and from 25 apparently healthy volunteers were enrolled in this study. Periodontal parameters used in this study were plaque index (PI), gingival index (GI), bleeding on probing (BOP), probing pocket depth (PPD) and clinical attachment level (CAL) and Serum levels of Anti-PC IgG was estimate by enzymelinkedimmunosorbent assays (ELISA) and hs-CRP was estimate by immunoturbidimetric.

Results: The current data revealed a significant elevation (p<0.01) in median level of hs-CRP in CP group and CP+ATH group in comparison to that in healthy control, moreover, the comparison between two groups of patients showed significant differences between two groups (p<0.05). Whereas the serum level of antiphosphorylcholine-IgG was not observed significant differences between study groups (p>0.05) except for CP group when compared to control group (p≤0.05). Moreover, in regards to the correlation between serum antiphosphorylcholine –IgG and hs-CRP, and clinical periodontal parameters, did not show any correlation with clinical parameters of periodontitis (p>0.05).

Conclusion: inflammatory mediator (hs-CRP) may increase inflammatory activity in atherosclerotic lesions and potentially increasing the risk for cardiovascular events in CP+ATH, furthermore, about the effect of Anti-PC IgG in ATH +CP group, further studies are needed to obtain more understanding result.

Key words: Atherosclerosis cardiovascular disease, chronic periodontitis, Antiphosphorylcholine, hs C-reactive protein.

Introduction

Periodontitis is described as a multifactorial, irreversible and cumulative condition initiated and propagated by both bacteria and host factors and is also associated with various systemic conditions.

Atherosclerosis and its cardiovascular ischemic complications are the most common causes of death and disability worldwide. It’s a chronic lipid-driven inflammatory disease of the arterial wall characterized by the involvement of the innate and adaptive immune systems. Inflammation plays a critical role in the atherosclerotic process, starting from endothelial dysfunction through all stages of plaque build-up until its detrimental clinical ischemic complications.
Associations between periodontitis and atherosclerosis would be predicted based on inflammatory mechanisms initiated by bacteria associated with periodontal lesions, locally or systemically, that then influence the initiation or propagation of the atherosclerotic lesion. Such lesions may be initiated by inflammatory stimuli including systemic and locally produced inflammatory cytokines and chemotactic agents that cause changes in the endothelium such as up-regulation of adhesion molecules. These changes promote interactions with leucocytes, such as monocytes, that promote leucocyte migration into the intimal layer of the artery (5).

C-reactive protein is an acute-phase reactant that is mainly produced in the liver in response to a variety of inflammatory cytokines such as IL-6. It therefore serves as a marker for systemic inflammation in a variety of conditions (6). Abd et al. reported that elevated cell- and cytokine mediated markers of inflammation, including C-reactive protein, fibrinogen, and various cytokines, are associated with periodontal disease. The same elevated proinflammatory factors in PD have also been linked with atherothrombogenesis (7).

Phosphorylcholine (PC) is an immunogenic epitope in the capsular polysaccharide of Streptococcus pneumoniae and it has been suggested that the presence of serum anti-PC is partly a result of exposure to this organism (8). However, periodontitis patients exhibit elevated levels of anti-PC and this antibody reacts with 30–40% of bacteria in dental plaque samples, including periodontitis-associated bacteria and is demonstrated by higher serum levels of antibodies directed toward phosphorylcholine (anti-phosphorylcholine IgG) in patients with attachment loss, in comparison with those with healthy gingiva (9).

Shaw et al., in 2003 suggests that antibodies produced against certain periodontal bacteria would also react to phosphorylcholine bearing oxLDL (10) and, therefore, magnify the uptake of this lipid by foam cells, promoting further progress of atherosclerosis.

**Results**

The demographic results of patients groups and controls group included in this study are presented in Table 1, there was male’s predominance among study groups when compared to females. Furthermore, the differences in clinical periodontal parameters in patients and healthy controls are summarized in Table 2.

The current results revealed a significant elevation in median serum level of hs-CRP in CP group (0.210 mg/dl) and in CP+ ATH patients (0.575 mg/dl) as compared to healthy control (0.180 mg/dl), (p<0.01). Moreover, the comparison between two groups of patients showed that the median level of hs-CRP was increase in CP group but statistically not significant (p>0.05). Whereas the serum level of antiphosphorylcholine-IgG was not observed significant differences between study groups (p>0.05) except for CP group when compared to control group (p≤0.05) as revealed in table 3. Furthermore, in regard to the correlation between serum hs-CRP and Anti-PC IgG, and clinical periodontal parameters did not show any correlation with clinical parameters of periodontitis (p>0.05) as shown in table 4.
### Table 1: Demographic characteristics of study group

<table>
<thead>
<tr>
<th>Gender and age</th>
<th>CP cases n=30</th>
<th>CP+ATH cases n=30</th>
<th>Controls n=25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male NO.</td>
<td>19</td>
<td>27</td>
<td>12</td>
</tr>
<tr>
<td>%</td>
<td>63%</td>
<td>90%</td>
<td>48%</td>
</tr>
<tr>
<td>Female NO.</td>
<td>11</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>%</td>
<td>37%</td>
<td>10%</td>
<td>52%</td>
</tr>
<tr>
<td>age Range</td>
<td>35-44</td>
<td>14</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>45-54</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>55-64</td>
<td>7</td>
<td>13</td>
</tr>
</tbody>
</table>

### Table 2: Clinical Periodontal Parameters in Study Groups

<table>
<thead>
<tr>
<th>Clinical periodontal Parameters</th>
<th>Study group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CP</td>
<td>CP + ATH</td>
</tr>
<tr>
<td>P.I</td>
<td>1.69(±0.36)</td>
<td>2.04(±0.29)</td>
</tr>
<tr>
<td>P.I</td>
<td>1.43(±0.27)</td>
<td>1.37(±0.23)</td>
</tr>
<tr>
<td>BOP</td>
<td>32.91%</td>
<td>40.54%</td>
</tr>
<tr>
<td>PPD</td>
<td>4.91(±0.590)</td>
<td>4.61(±0.601)</td>
</tr>
<tr>
<td>CAL</td>
<td>4.51(±0.741)</td>
<td>5.09(±1.115)</td>
</tr>
</tbody>
</table>

** = Highly significant difference (p=0.0001), *=significant difference

### Table 3: The differences in median serum levels of hs-CRP (mg/dl) and Antiphosphorylcholine-IgG (u/ml) among study groups.

<table>
<thead>
<tr>
<th>Immunological Parameters</th>
<th>CP</th>
<th>CP+ATH</th>
<th>control</th>
<th>P-value (Kruskal Wallis test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hs-CRP</td>
<td>Mean rank</td>
<td>40.65</td>
<td>53.45</td>
<td>33.28</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>0.210</td>
<td>0.575</td>
<td>0.180</td>
</tr>
<tr>
<td>Anti-PC IgG</td>
<td>Mean rank</td>
<td>46.08</td>
<td>46.83</td>
<td>34.79</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>1.525</td>
<td>1.700</td>
<td>1.250</td>
</tr>
</tbody>
</table>
### Table 4: Spearman Correlation Coefficients among serum levels of (hs CRP and Anti-PC IgG) and clinical periodontal parameters in ATH+CP group and CP group

<table>
<thead>
<tr>
<th>Immunological Parameters</th>
<th>Groups</th>
<th>Statistical analysis</th>
<th>P.I</th>
<th>GI</th>
<th>BOP</th>
<th>PPD</th>
<th>CAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CP</td>
<td>r</td>
<td>.330</td>
<td>.152</td>
<td>.256</td>
<td>.108</td>
<td>.327</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p-value</td>
<td>.075NS</td>
<td>.424NS</td>
<td>.171NS</td>
<td>.570NS</td>
<td>.078NS</td>
</tr>
<tr>
<td></td>
<td>CP+ATH</td>
<td>r</td>
<td>.084</td>
<td>.068</td>
<td>.108</td>
<td>.141</td>
<td>.176</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p-value</td>
<td>.659NS</td>
<td>.720NS</td>
<td>.571NS</td>
<td>.458NS</td>
<td>.352NS</td>
</tr>
<tr>
<td></td>
<td>CP</td>
<td>r</td>
<td>.088</td>
<td>-.102</td>
<td>-.128</td>
<td>-.113</td>
<td>.251</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p-value</td>
<td>.644NS</td>
<td>.593NS</td>
<td>.502NS</td>
<td>.551NS</td>
<td>.181NS</td>
</tr>
<tr>
<td></td>
<td>CP+ATH</td>
<td>r</td>
<td>-.186</td>
<td>.099</td>
<td>.090</td>
<td>-.020</td>
<td>-.073</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p-value</td>
<td>.325NS</td>
<td>.605NS</td>
<td>.636NS</td>
<td>.916NS</td>
<td>.702NS</td>
</tr>
</tbody>
</table>

**Discussion**

The possible association between periodontitis (PD) and atherosclerotic cardiovascular disease (ASCVD) has received much attention over the past two decades. A comprehensive review was performed by an American Heart Association (AHA) working group (11), which concluded that “periodontal disease is associated with atherosclerotic vascular disease independent of known confounders”.

In the present study a predominance of CP group among males than a female as male less attitude toward good oral hygiene (12) as well as in ATH+CP as the Estrogen is protect female as it has multiple effects including effects on lipids, nitric oxide, vascular tone and antioxidant properties (13).
The current result was found that the mean value of (P.I, G.I, and CAL) were higher in ATH+CP group as compared to CP group which is in accordance with the observations of the Kumar et al.\(^{(14)}\), as they found that there was significant increase in mean of periodontal parameter (P.I, G.I) in ATH with CP patient compared to healthy control, Similarly Mohamad et al.\(^{(15)}\) found that the mean value of each P.I, G.I were significantly higher in periodontitis patients when compared to healthy controls, while Thakare et al.\(^{(16)}\) Deepa et al.\(^{(17)}\) found that CAL was more in ATH with CP as compared to CP group. The possible explanation the presence of dental plaque is the main clinical finding for CP and it is coincide with the severity of the disease and the time being with the disease and it is expected to be accumulating more in CP, another possible explanation of such results as the hospitalized ASCVD patients neglect the oral hygiene measures and didn’t brush their teeth regularly. In addition, CAL indicates the amount of the root surface denuded of periodontal attachment, thus it was used as an indicator of disease severity. The percentage of score 1 of BOP sites and CAL demonstrated by CP group were higher when compared with ATH+CP group, and these result was consistent with Androsz-Kowalsa et al.\(^{(18)}\) and Raheem et al.\(^{(19)}\) they found mean bleeding index in chronic periodontitis was significantly higher than in CAD with chronic periodontitis and control. The possible explanation for that percentage of BOP sites demonstrated by CP group was higher when compared with ATH+CP group, may be due to the presence of more inactive sites during clinical periodontal examination of ATH+CP group, also in CP the examining sites more than in ATH+CP group so ASCVD might be lead to tooth loss.

The present work is found increase in serum levels of hs-CRP in CP+ATH patients when compared to CP and control groups which is in accordance with the observations of the previous researchers\(^{(16,20)}\). The possible explanation for positive association existed between the presence of chronic periodontitis and high serum CRP levels because it is biologically plausible that inflammatory mediators, especially IL-1 and -6 and TNF-a are released under conditions of periodontitis and present the capacity to stimulate hepatocytes to produce CRP\(^{(21)}\). In addition direct actions of CRP which contribute to the induction of a prothrombotic state may be the enhancement of the procoagulant activity\(^{(22)}\) on the other hand, regarding IgG antiphosphylcholine revealed significant difference between CP group when compared to control group and these result in agreement with\(^{(9,23)}\) who found that patients with periodontal attachment loss have higher concentrations of anti-PC IgG than do individuals who demonstrate no attachment loss. The possible explanation for the higher level of serum IgG anti-phosphoryl-choline antibodies among CP patients in our study that inflamed periodontal tissues permit ingress of antigens from oral bacteria which leads to increased systemic production of anti-PC\(^{(23)}\).

Regarding the correlation between serum (hs-CRP and anti-PC IgG) and clinical periodontal parameters, hs-CRP did not show significant correlation. And these was agreed with Ide and co-workers reported that there were no correlation between serum hs CRP and clinical periodontal parameters and they concluded that improvement in periodontal health status by non-surgical periodontal treatment did not influence the serum levels of hs CRP\(^{(24)}\), in contract were inconsistent to that reported by others investigators \(^{(16,25)}\) they found that there was significant positive correlation between serum CRP levels and clinical periodontal parameters in ATH with CP and CP group. Finally, antiphosphoylcholine IgG did not show significant correlation with clinical periodontal parameters and our result disagreed with Schenkein et al.\(^{(11)}\) as they reported higher serum levels of antibodies (antiphosphorylcholine IgG) directed toward phosphorylcholine in patients with attachment loss, in comparison with those with healthy gingiva. The discrepancies observed between various studies could be caused, in part, to the differences in the sample size of each study, differences in types of samples used for each study and differences in sampling methods.

In conclusion current results suggest that elevation in inflammatory mediators (hs-CRP) may increase inflammatory activity in atherosclerotic lesions and potentially increasing the risk for cardiovascular events in CP patients. While for Anti-PC IgG the data show that many commonly occurring bacterial species found in dental plaque contain PC antigen and as a consequence of inflammation and periodontal attachment loss may influence systemic anti-PC antibody concentration, and on his role in atherosclerosis cardiovascular disease, further studies are needed to obtain more understanding result.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.
Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

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The Diagnostic Test in Lumbosacral Vertebrae Image towards Magnetic Resonance Imaging in Radicular Low Back Pain

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¹Department of Neurology, ²Department of Radiology, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo Teaching Hospital, Surabaya 60285, Indonesia

Abstract

Background: Lower back pain is one of the most common consulted cases in neurology and neurosurgery. The approximately 80% of these cases are musculoskeletal disorders. Magnetic resonance imaging (MRI) is still a rare examination in the suburban area and requires a relatively high cost. Moreover, there are contraindications in patients who have cardiac pacemakers and metal objects in the body. The examination of lumbosacral image is easy to conduct. The price is relatively inexpensive and there are no contraindications to perform the test. Objectivity: This study aimed to assess the sensitivity and specificity of the lumbosacral image towards MRI in patients with radicular lower back pain. Method: The study was a clinical, cross-sectional study, analytical, and a diagnostic test design. It was performed on all patients with radicular lower back pain in Dr. Soetomo Teaching Hospital Surabaya from April to July 2012. There were 34 subjects who fulfilled the inclusion criteria. Results: The sensitivity of plain images to MRI in detecting radicular lower back pain was 87.5%. This figure indicated that the plain image had a high sensitivity. However, the specificity was only 50.0% which meant that plain image had a low specificity. Conclusion: The lumbosacral image had high sensitivity. However, the specificity was low for the examination of radicular lower back pain.

Keywords: diagnostic test, radicular lower back pain, sensitivity, specificity.

Introduction

Lower back pain (LBP) is one of the most heavily consulted cases in neurology and neurosurgery unit and approximately 80% is musculoskeletal disorders. Most patients who complaint of acute LBP will experience relapse after healing. LBP complaints can disturb daily activity and work productivity as well as most often attack the productive age which is less than 45 years ¹-³. The prevalence of LBP is about 67.6% of the entire adult population per year in Australia, 42.6% of whom have mild LBP and mild disability; Another 10.9% had severe back pain and mild disability, while the remaining 10.5% had severe disability ⁴. The mean prevalence of lower back pain in African teenagers was 12%, and adult age was 32% ⁵. The estimated prevalence of lower back pain in the United States is about 5-20% and around 25-45% of the population in Europe ⁶. Although epidemiologic data on LBP is not observed yet in Indonesia, it is estimated that 40% of Central Java residents aged between 65 years old have suffered from back pain and its prevalence in males was 18.2% and 13.6% in females. LBP is one of the ten causes of patients visiting a doctor. The cause of the pain is often not found even with neuroimaging test; thus, the patient returns with an idiopathic LBP diagnosis ².

One of the LBP diagnostic support tools is radiological examination. The best radiological modality to evaluate herniated disc is MRI with sensitivity and specificity of 96% and 97%. MRI has sensitivity of 89% - 100% whereas the specificity was 43% - 57%. MRI is the golden standard of herniated disc. Besides that, MRI can detect soft tissue abnormalities (muscles, tendons, and ligaments) as well as edema that occurs around herniation nucleus pulposus (HNP) and detect other serious disorders such as tumors or infections ⁷. MRI is still a rare examination in the suburban area.
and requires a relatively high cost. Moreover, there are contraindications in patients who have cardiac pacemakers and metal objects in the body. The examination of lumbosacral image is an easy task to be conducted because almost all local hospitals have conventional x-ray aircraft and they are relatively inexpensive. Furthermore, there is no contraindication to conduct such examination.

Based on several previous studies of lumbosacral bone radiographic assessment of 200 patients with non-specific back pain and no neurological signs, it suggests that radiographic examination of the lumbosacral vertebra is adequately represented by a projection that is only antero-posterior or lateral to reduce the risk of radiation. The National Institute of Clinical Studies Emergency Care Community of Practice issued a recommendation that routine radiological examination is not recommended for non-specific acute lower back pain (level III-2 evidence) in May 2007. Similarly, a study published by the British Journal of General Practice in May 2002 examined the psychological effects of lower back pain patients who performed radiological examination (X-ray photograph of the lumbar vertebra) at the beginning of LBP diagnosis established.

This study attempted to re-examine the diagnostic test of lumbosacral image towards MRI in radicular lower back pain patients to assess the sensitivity and specificity of the lumbosacral plain image towards the lumbosacral MRI. In addition, a neurological clinical examination has been performed to determine the diagnosis of radicular lower back pain.

**Method**

The study was a clinical, cross-sectional study, analytical, and a diagnostic test design. The sample collection was conducted in Neurology Unit dr. Soetomo Teaching Hospital Surabaya for 3 months. The research subjects are 46 patients with radicular low back pain who visited the neurological clinic Dr. Soetomo Teaching Hospital Surabaya, Indonesia and willing to fill and sign the informed consent. Then, a neurologic clinical examination was performed: anamnesis, provocation test, physiological and pathological reflex tests, motor, and sensory tests. Afterwards, it was examined a plain radiograph (Rontgen) in lumbosacral vertebrae of antero-posterior, lateral, and oblique projection after receiving description of the advantages and disadvantages of the photograph. Afterwards, a lumbosacral MRI was examined. The results of the examination were observed whether the abnormality was obtained or not.

The data was collected on the data collection sheet and the measurement result was expressed on the dichotome scale which was the nominal scale of positive and negative. The clinical examination results of lower back pain with radiological examination was analyzed using SPSS program (SPSS, Inc., Chicago, IL). The differences in the number of positive clinical and radiological findings were compared with the negative findings; then, it was also compared to MRI.

**Results**

Overall, there were 17 subjects of female patients and 17 male subjects. The subjects had the same complaints of radicular lower back pain or lower back pain that spreads to the back of the leg. There were 17 samples of males (50%) and 17 female samples (50%) with a ratio of 1:1 in this study. Table 1 described the characteristics of research subjects based on gender.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Total</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>17</td>
<td>50%</td>
</tr>
<tr>
<td>Male</td>
<td>17</td>
<td>50%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age (year)</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-30</td>
<td>5</td>
<td>14.7</td>
</tr>
<tr>
<td>31-40</td>
<td>6</td>
<td>17.6</td>
</tr>
<tr>
<td>41-50</td>
<td>12</td>
<td>35.3</td>
</tr>
<tr>
<td>51-60</td>
<td>5</td>
<td>14.7</td>
</tr>
<tr>
<td>61-70</td>
<td>4</td>
<td>11.8</td>
</tr>
<tr>
<td>&gt;71</td>
<td>2</td>
<td>5.9</td>
</tr>
</tbody>
</table>
Table 3. The Subject Characteristics Based on Mean Age

<table>
<thead>
<tr>
<th>Variable</th>
<th>Subjects (34)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Minimum</td>
<td>Maximum</td>
</tr>
<tr>
<td>Age (year)</td>
<td>46.03</td>
<td>1.38</td>
<td>22</td>
<td>71</td>
</tr>
</tbody>
</table>

Table 2 showed the age of the subjects in the study. The most frequent age of the subjects was 41-50 years old that was 12 times (35.3%). Table 3 showed that the mean age was 46 years old (SD = 1.38) with the youngest age was 22 years old and the oldest was 71 years old. The mean morbidity measured by visual analogue scale (VAS) was 7 (SD = 1.164) with a minimum score of 5 and a maximum of 9.

Table 4. The Subject Characteristics Based on the Frequency of VAS for Pain

<table>
<thead>
<tr>
<th>VAS</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>3</td>
<td>8.8</td>
</tr>
<tr>
<td>6</td>
<td>11</td>
<td>32.4</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>29.4</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>17.6</td>
</tr>
<tr>
<td>9</td>
<td>4</td>
<td>11.8</td>
</tr>
</tbody>
</table>

Table 4 showed the severity of pain measured by VAS which was most often suffered in the scale of 6 which was moderate-severe scale. The scale 7 and 8 were categorized as severe pain. The onset of the subjects complained of radicular low back pain until the examination in Neurology Unit Dr. Soetomo Teaching Hospital was in a matter of weeks. Table 5 showed the most commonly seen subjects with an onset of more than 24 weeks, in other words, they already suffered from chronic LBP.

Table 5. The Subject Characteristics Based on the Disease Onset

<table>
<thead>
<tr>
<th>Onset (week)</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>5</td>
<td>14.7</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>2.9</td>
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<td>8</td>
<td>9</td>
<td>26.5</td>
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<td>10</td>
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<tr>
<td>12</td>
<td>6</td>
<td>17.6</td>
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<tr>
<td>&gt;24</td>
<td>11</td>
<td>32.4</td>
</tr>
</tbody>
</table>
Table 6. The Result of Plain Image Compared to MRI

<table>
<thead>
<tr>
<th>Disorder</th>
<th>MRI</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>Plain Image</td>
<td>There is disorder: 28</td>
<td>1 29</td>
</tr>
<tr>
<td></td>
<td>Normal: 4</td>
<td>1 5</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>2 34</td>
</tr>
</tbody>
</table>

Note: MRI = Magnetic Resonance Imaging

Table 6 showed the sensitivity of plain images to MRI in detecting radicular lower back pain by 87.5%. This figure indicated that the plain image had a high sensitivity. However, the specificity was only 50% which meant that plain image had low specificity.

**Discussion**

This study obtained a description of the lumbosacral plain photographs, including osteophytes, end plate sclerosis, narrowing of the intervertebral gap, and spondylolisthesis, that is corresponding to MRI examination results: bulging intervertebral disc, protrusion, extrusion, causing radicular lower back pain or radiating from the root to the length of the sciatic nerve. This condition is diagnosed as herniation nucleus pulposus (HNP). Another finding in this study by examining the lumbosacral plain photograph is the description of the destruction of the corpus, vertebral pedicles and scalloping features. This corresponds to the results of MRI examination in the form of tumor masses, both metastases and primary tumors in the lumbosacral area.

The characteristics of the subjects by gender in this study obtained the same number of male and female that was 17. This is not in accordance with the epidemiology of lower back pain that stated that 40% of Central Java residents aged 65 years old have suffered from lower back pain and the prevalence in males was 18.2% and in women was 13.6% 14. The cause of this discrepancy can be some of the epidemiological shifts of most males with lower back pain. However, females mostly cause of the vast majority with LBP. They consisted of factory and office workers. Another reason was that the most frequent LBP patients who visited Neurology Unit in Dr. Soetomo Teaching Hospital was males than females. The subjects who experienced a drop out in this study was 12 male subjects. In addition, the most frequent age was 41-50 years old. This is consistent with the epidemiological theory that the majority of LBP patients are of productive age because their load is also greater. LBP complaint disturbs daily activity and work productivity as well as most often attacks the productive age which is less than 45 years old 14.

The most frequent onset was more than 24 weeks and categorized as chronic. It should be distinguished between specific lower back pain and nonspecific. Because this study only focused on the specific LBP subjects, most of them are in chronic condition that conventional or medical treatment was usually not solve the problem and recurrence often occurs. Unlike the nonspecific LBP types that are mostly due to musculoskeletal or mechanical factors, the complaint can be relieved usually by 2 to 6 weeks of therapy according to the severity of pain 1,15.

Table 3 showed the sensitivity of the lumbosacral plain image compared to the lumbosacral MRI (as the golden standard) as the figure was 87%. The specificity of the lumbosacral plain photograph was 50%. This corresponds to the literature: a radiographic examination of a plain photograph to detect lumbosacral vertebral abnormality has a sensitivity of about 85% and a specificity of approximately 60% 16. Thus, the hypothesis of this study aims to know the examination of plain images of the lumbosacral vertebra has a high sensitivity and specificity towards the lumbosacral MRI in patients with radicular lower back pain has been proven for the sensitivity of the lumbosacral plain photographs, but not for the low specificity.

The sensitivity here illustrates the sensitivity of the lumbosacral plain photograph as a diagnostic tool in detecting a radicular lower back pain, whether the subjects are really sick or not, and how likely it is that
the outcomes are positive or there are abnormalities. This sensitivity rate is derived from the proportion of the patients with positive diagnostic test results (true positive) over all patients (true positive + false negative). On the other hand, the specificity is the ability of the lumbosacral plain photograph to determine that the subject is not sick or normal and indicates how much probability the test result will be negative. In the results of this study, the positive predictive value of 96.6% is categorized as high. This means that if the subjects were examined with a plain lumbosacral image with a positive result, then 96.6% of the subjects suffered from a disease that causes radicular lower back pain. The negative predictive value is the probability that the subject does not suffer from the disease if the test result is negative. In this study, the results of negative predictive value of 20% indicates that there is a possibility of 20% of subjects who have been examined with plain lumbosakral image which results are negative and not suffer from disease that causes radicular lower back pain.

The interpretation of the diagnostic test result is influenced by several things, particularly the prevalence of disease and the severity of disease at the time of diagnostic testing. Other things to consider in assessing the accuracy of lumbosacral plain photographs include lateral and oblique positions are often confused by the iliac crest and gas image in the intestine and influenced by the accuracy of the radiographic technique as well as the radiologist’s skill. The weakness of plain photographs cannot detect abnormalities in the ligaments, muscles, nerves, and other soft tissues around the bone. A plain photo sensitivity of 85% and a specificity of 60% only applies to detect solid organs such as bone. Generally, neuroimaging has advantages as well as deficiencies that should be complementary, such as plain photographs (conventional radiography) and CT-scans have advantages in detecting abnormalities of solid organs such as bone and calcification.

On the other hand, MRI has more ability in detecting abnormalities in the soft tissues. Besides that, MRI can be drawn by multiple slices which can be rotated electronically on several orientations without changing the patient’s position. MRI is also non-ionic and non-invasive; thus, it does not have radiation effects on body tissues and make patients feel uncomfortable during the examination. However, MRI has several disadvantages, including hearing loss can occur temporarily due to the noise of this tool during the examination. A research showed a loss of hearing <15dB when the subjects did not wear earplugs in 43% patients, whereas only 10% when the subjects used earplugs with an average length exposed to the sound for 42.1 minutes. Furthermore, there may be an effect of increased temperature of the network by radio frequency absorbed of <1 W/kg. Ventricular fibrillation also can occur due to the influence of the magnetic field. This situation is influenced by the length of the pulse radiofrequency (RF) signal and repetition. It is stated that frequency >60Hz decreases the threshold value of ventricular fibrillation. MRI has a low sensitivity in detecting sub-arachnoid classification and bleeding and not provide comfort in patients with claustrophobia.

**Conclusion**

The lumbosacral image had high sensitivity (87.5%). However, the specificity was low for the examination of radicular lower back pain (50.0%).

**Ethical Clearance:** This study protocol was approved by ethical clearance Dr. Soetomo Teaching Hospital Surabaya, Indonesia.

**Conflict of Interest:** The author reports no conflict of interest of this work.

**Source of Funding:** This study is done with individual funding.

**References**

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Study of Gastroesophageal Reflex Disease in adult Type II Diabetes Mellitus Patients with Upper Gastrointestinal Symptoms

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Abstract
Gastrointestinal symptoms are relatively common in clinical practice in patients with type II diabetes mellitus. type II DM has been described as possible risk factor for the development of gastroesophageal reflux disease, in this study we aim to detect the prevalence of GRED in symptomatic patients with type II DM , also to see the accuracy of reflex disease questionnaire ( RDQ) in the diagnosis of GERD in patients with type II DM in relation to the esophagogastroduodenoscopy finding and to study the relation of autonomic neuropathy to the prevalence and stages of GERD in diabetic patients .

Methods: A ninety patients with upper gastrointestinal symptoms was divided in to tow groups according to whether had type II DM or not and then each group involved in a two stage process ( a ) – a RDQ , ( b ) – OGD.

And patients in the DM group underwent another step to detect diabetic neuropathy by a bed side clinical test.

Results: The prevalence of esophagitis in this study was higher in the DM group. RDQ had a statistical significance in detecting esophagitis in the DM group. There is no relation of autonomic neuropathy to the prevalence of GERD in diabetic patients .

Conclusion : The prevalence of GERD was high in patients with type II DM, RDQ is a sensitive tool for the diagnosis of GERD in diabetic patients, Autonomic neuropathy did not increase prevalence of esophagitis in diabetic patients .

Keywords: diabetes mellitus ,gastro esophageal reflex disease , RDQ, Esophagogastroduodenoscopy, Esophagitis.

Introduction
The current concept of GERD is “symptoms or complications associated with regurgitation from the stomach and or the duodenum to the esophagus” It is estimated that 15%-30% of the general population are affected by GERD(1,2)

GERD develops when the esophageal mucosa exposed to gastroduodenal contents for prolonged periods of time, resulting in symptoms and, in a proportion of case , esophagitis(2).

Note that some degree of gastroesophageal reflux is normal, physiologically connected with the mechanism of belching (transient LES relaxation), but esophagitis results from excessive reflux, often accompanied

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by impaired clearance of the refluxed gastric juice. Restricting reflux to that which is physiologically intended depends on the anatomic and physiologic integrity of the oesphagogastric junction, a complex sphincter comprised of both the LES and the surrounding crural diaphragm. Three dominant mechanisms of esophagogastric junction incompetence are recognized: (a) transient LES relaxations (a vagovagal reflex in which LES relaxation is elicited by gastric distention), (b) LES pressure decrease, or (c) anatomic distortion of the esophagogastric junction inclusive of hiatus hernia (1,2).

Transient LES relaxations account for at least 90% of reflux in normal subjects or GERD patients without hiatus hernia, in many studies the first two mechanisms and other factors such as abdominal obesity, delayed gastric emptying, disruption of esophageal peristalsis, autonomic neuropathy, metabolic syndrome were thought to be a possible risk factors for the high prevalence of the typical GERD symptoms in patient with DM, but up to present, the pathogenesis of GERD in type II DM patients has not been fully clarified (3,4,5).

Peripheral and autonomic neuropathy especially has become a research hotspot in recent years, however, the impact of the above factors on the presence of GERD symptoms in type II DM patients is still under debate (3,6).

Patients with GERD may present with heartburn and regurgitation which are the major symptoms of GERD, somewhat less common are dysphagia and chest pain and a variety of other features has been described, and depending on this a clinical system of symptom-based diagnosis of GERD was made the so called reflex disease questionnaire RDQ (7).

RDQ comprises 6 questions assessing the frequency of heartburn, substernal chest pain, acid regurgitation, nausea, need for the medication, and trouble getting a good night sleep because of heartburn or regurgitation,per week, as shown in table 1.

**Table 1: GerdQ Questionnaire**

<table>
<thead>
<tr>
<th>Symptom</th>
<th>How Many Times Does This Occur Per Week?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Days</td>
</tr>
<tr>
<td>Burning feeling behind the breastbone (heartburn)</td>
<td>0</td>
</tr>
<tr>
<td>Stomach contents moving up to the throat or mouth (regurgitation)</td>
<td>0</td>
</tr>
<tr>
<td>Pain in the middle of the upper stomach area</td>
<td>3</td>
</tr>
<tr>
<td>Nausea</td>
<td>3</td>
</tr>
<tr>
<td>Trouble getting a good night’s sleep because of heartburn or regurgitation</td>
<td>0</td>
</tr>
<tr>
<td>Need for over-the-counter medicine for heartburn or regurgitation (such as Tums, Rolaids, Maalox, or other antacids), in addition to the medicine your doctor prescribed</td>
<td>0</td>
</tr>
</tbody>
</table>

**NOTE:** Add the point values for each corresponding answer. Total score of 0 to 2 points = 0 percent likelihood of GERD; 3 to 7 points = 50 percent likelihood; 8 to 10 points = 79 percent likelihood; 11 to 18 points = 89 percent likelihood.
Several investigation used to evaluate patients with suspected having GERD of which endoscopy is the initial investigation of choice, it used for the diagnosis and grading of GERD and to exclude other upper gastriointestinal disease that can mimic GERD and to identify complications.

**Aim of the Study**

To study the prevalence of GERD in symptomatic patients with type II DM in relation to non DM, also to see the accuracy of RDQ in the diagnosis of GERD in patients with type II DM in relation to the OGD finding and to study the relation of autonomic neuropathy to the prevalence of GERD in diabetic patients present with upper gastro intestinal symptoms.

**Patients and Method**

This study was conducted in Alsader medical city, in Alnajaf center for gastrointestinal and hepatic diseases in which 122 adult patients referred for upper endoscopy because of upper gastrointestinal symptoms suggestive of GERD between March 2014 and March 2015 participate in this study.

Exclusion criteria were type I DM, esophageal motility disorder other than ineffective esophageal motility, patient with hiatus hernia and patient with a RDQ less than 8.

After performing the exclusion criteria a 90 patients were considered eligible for this study 31 of them were females and 59 were males.

The recruitment patients were divided into two groups according to whether had a type II DM or not, the diagnosis of type II DM based on WHO definition (Defined as fasting blood glucose $\geq$ 7 mmol/l or on glucose medication for raised blood glucose or with a history of diagnosis of diabetes.)

The then every group involved in a two stage process (a) Reflux disease questionnaire (RDQ) and patients with at least a minimal score of 8 had been chosen as the (RDQ) likelihood of GERD were as fellow:

1. A total score 0 to 2 = 0 percent likelihood
2. A total score 3 to 7 = 50 percent
3. A total score 8 to 11 = 79 percent
4. A total score 12 to 18 = 89 percent.

(b) OGD by the use of Olympus or pentax ED-3490 TK 4.2 HOYA corporation endoscopy system, which used in the diagnosis and staging of esophagitis according to Savery-Miller classification in these patients.

(c) the patients with DM group underwent another step during interview by being underwent a simple clinical test to detect the diabetic autonomic neuropathy (a bedside test for the absence respiration-associated sinus arrhythmia by monitoring the pulse rate by a pulse oximeter while the patient instructed to breathe slowly and deeply at a rate of 6 breaths per minute, heart rate normally increase with inspiration and decrease with expiration, and at a respiratory rate of 6 per minute the difference between fastest and slowest heart rate is usually more than 15 per minute, a difference of 10 beats per minute or less are observed in autonomic dysfunction.

**Statistical Analysis**

Statistical analysis were performed using spss 16 program correlation analysis was also performed.

A P value $<0.05$ was considered significant.

**Results**

Ninety patients were included in this study and divided in to two groups a thirty patients in DM group and sixty patients in the non DM group.

In the non DM group 22 of them were females(36.66%) and 38 were males(63.33%) with a mean age of (46.7 years±8.2).

In the DM group 9 were females (30%) and 21 were males (70%) with a mean age of (51.2 years±9.3). As shown in table 2.

**Table-2 the demographic characters of both groups (DM and non DM)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>Non DM</td>
<td>22(36.66%)</td>
</tr>
<tr>
<td>DM</td>
<td>9(30%)</td>
</tr>
<tr>
<td>Male</td>
<td></td>
</tr>
<tr>
<td>Non DM</td>
<td>38(63.33%)</td>
</tr>
<tr>
<td>DM</td>
<td>21(70%)</td>
</tr>
<tr>
<td>Mean age years</td>
<td>Non DM 51.2±9.3</td>
</tr>
<tr>
<td></td>
<td>DM 46.7±8.2</td>
</tr>
</tbody>
</table>
The prevalence of esophagitis in this study were higher in the DM group as upper OGD results showed esophagitis in 32 patients of the non DM group (53.3%) and in 23 patients of the DM group(76.66%) with a statistical significant results (p value=0.03236) as shown in table 3.

<table>
<thead>
<tr>
<th>Group</th>
<th>Esophagitis</th>
<th>Percentage</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non DM</td>
<td>32 of 60</td>
<td>53.33%</td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>23 of 30</td>
<td>76.66%</td>
<td>0.03236*</td>
</tr>
</tbody>
</table>

**Table 3: (the prevalence of esophagitis by OGD in both study groups)**

In comparing the RDQ which about 80% in a score level 8-11 and OGD result in detecting esophagitis in both groups the result shows that RDQ had a statistical significance in detecting esophagitis in the DM group as shown in table 4.

<table>
<thead>
<tr>
<th></th>
<th>By QRS 80%</th>
<th>By OGD</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients in DM group with esophagitis</td>
<td>24 of 30=80%</td>
<td>23 of 30=76.6%</td>
<td>0.00244*</td>
</tr>
<tr>
<td>Number of patients in non DM group with esophagitis</td>
<td>48 of 60=80%</td>
<td>32 of 60=53.3%</td>
<td></td>
</tr>
</tbody>
</table>

In the DM group the clinical bedside test for autonomic neuropathy revile an autonomic dysfunction in 16 patients of 30 (53.33%) and 14 patients show not (46.6%).

13 (81.2%) of the 16 patients with DM and autonomic dysfunction were diagnosed with esophagitis by OGD and 3 patients show no sign of esophagitis by OGD(18.7%), while in patients with DM and without autonomic dysfunction there were 10 patients of 14 (71.4%) diagnosed with osephagitis by OGD and 4 patients(28.5%) are not, so there were no statistical significance P value=0.5287 as shown in table 5.

**Table 5-prevalence of esophagitis in DM patients with and without autonomic dysfunction**

<table>
<thead>
<tr>
<th>Patient in DM group</th>
<th>Patient with esophagitis</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>With autonomic dysfunction no=16</td>
<td>13 of 16(81.2%)</td>
<td>0.5287</td>
</tr>
<tr>
<td>Without autonomic dysfunction no=14</td>
<td>10 of 14(71.4%)</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

Diabetes is a major health problem, as it is an important contributor to various other diseases, and its incidence still continues to rise. Gastrointestinal symptoms are relatively common in clinical practice in patients with type II DM. Type II DM has been described as possible risk factor for the development of GERD. Several studies reported that the prevalence of GERD symptoms in the type II DM was approximately 25%-40% in different populations\(^\text{11,12,13}\), in these studies several methods used in the diagnosis of GERD includes; RDQ which had a sensitivity of 79% in the diagnosis of GERD at 8-11 score level in non DM patients; Endoscopic examination which had high specificity but low sensitivity for the diagnosis of GERD with a sensitivity about 55% for detecting esophagitis and other complications\(^\text{14,15}\) and ambulatory PH monitoring which is indicated if diagnosis is unclear or surgical intervention is under consideration, although the test yields accurate and reliable information it is inconvenient for the patient and many clinicians do not have access to the appropriate instrumentation and are unable to perform this test\(^\text{16}\).

In this study a combination of RDQ and OGD is used to study the prevalence of GERD in patients with type II DM as a previous study by Lemeneh Tefera, B.A., Martin Fein.\(^\text{16}\) shows that this combination had a high proof value 98% and high specificity 97% for the diagnosis of GERD in non DM patients, in this study we found that the prevalence of GERD in adult symptomatic patient with type II DM as diagnosed by OGD were high 76.7% comparing to non DM patients 53.3% with a P value =0.03236.

We also found that the RDQ at level score >8 had a good prediction rate for esophagitis which about 80% in symptomatic patient with type II DM as the OGD result showed esophagitis in 76.66% in patients with type II DM comparing to non DM patients in regard to the sensitivity of RDQ with a P value=0.00244. and this was constant with a study by Yu Bai, Yiqi Du, Duowu Zou, Zhendong Jin and others, that suggest that the RDQ may be used for the diagnosis of GERD\(^\text{17}\).

In previous studies neuropathy were thought of as possible risk factor for the prevalence of the typical GERD symptoms in DM patients (13,20), for example, in 2008, Wang et al.\(^\text{13}\) reported that the prevalence of GERD symptoms was higher in patients with neuropathy than in patients without neuropathy, in this study there were no significant difference in the prevalence of esophagitis in DM patients with or without neuropathy with a P value=0.05287, this results are similar to the studies of Clouse and Lustman \(^\text{21}\) and Lee et al. \(^\text{19}\), In 1989, Clouse and Lustman, through analyzing 114 diabetic subjects with gastrointestinal motor dysfunction symptoms, reported that gastrointestinal symptoms occurring in diabetic patients were poorly related to neuropathic complications. In 2011, Lee et al.\(^\text{19}\) studied 119 patients with type II DM and found that there was no significant difference in the proportions of patients experiencing typical GERD symptoms between the two groups of type II DM with and without neuropathy.

Conclusion

The Prevalence of GERD were high in patients with type II DM, RDQ is a sensitive tool for the diagnosis of GERD in those patients, Autonomic neuropathy did not increase prevalence of esophagitis in diabetic patients.

Conflicts of Interest: None of the authors have any conflicts of interest relevant to this research subject.

Ethical Clearance: the study was conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki. The study protocol, care of patients and subject information were reviewed and approved by a local Ethic committee.

Source of Funding: Self

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The Effect of Health Information Technology on Time and Cost Saving in Remote areas of Iran

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Abstract

The present study aims at investigating the effect of health information technology on time and cost saving in the perspective of users of teaching hospitals in in remote areas of Iran.

This is an applied study with a descriptive-analytical and cross-sectional method conducted on users of hospitals in remote areas of Iran using stratified random sampling. Data collection instrument was a self-made questionnaire, the face validity and reliability of which were confirmed by Cronbach’s alpha 0.94. Data were collected in person and analyzed by SPSS software V. 21 and descriptive and analytical statistics.

Based on the Chi-square test results, there was a significant relationship between the effects of health information technology on time saving as well as cost saving. The results of Kruskal-Wallis test showed no significant relationship between education level, age and work experience and time and cost saving as well.

User satisfaction is one of the most important aspects of the success of information systems, therefore, it is recommended that senior managers pay attention to the individual aspects of the employees working in the organization, provide them with necessary training before implementing the information systems, and involve them in the decisions in using such technologies.

Keywords: Health information technology, time saving, cost saving, remote areas

Introduction

The application of information technology¹ in organizational activities is one of the most important reasons that distinguishes the organizations. As such, organizations that use IT widely and optimally have a sustained competitive advantage and are more distinguished in the perspective of stakeholders.¹ Obviously, IT plays a significant role in organizations. It can enhance the capabilities of organizations and reduce costs.²

Based on the literature, it is expected that in health care organizations, using health information technology² can improve the quality of health services, prevent medical errors, optimally manage health care costs, increase management efficiency, improve productivity, and reduce paperwork.³ However, in practice, according to the results of various studies, using HIT can have different and sometimes contradictory effects. For example, the results of some studies indicate the positive effect of technology on enhancing the quality of documentation, frequency, accuracy and completeness of nursing diagnosis, quality of health services, preventing medical errors, managing costs and increasing efficiency⁴, 5, 6, 7 & 8. In addition, people who experienced numerous failures in using the clinical information system were less inclined to the new information system. They believe that the benefits of HIT depends on the type of technology used and on how the technology is applied⁹, 10, 11, 12, 13, 14, 15, and 16. Nowadays, the tendency to use health information systems has grown dramatically as the role of information

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in the field of health care delivery becomes more important, but clearly, there is no clear answer to the question that how much investment and spending costs on this issue improves the quality and reduces cost of health care services and more profitability for health care providers and physicians. Responding to this ambiguity will convert the IT concept as a costly plan into a means of achieving value added, and prevents ambiguities that delay or even prevent some centers from investing in IT, and considering the importance of accurate and timely provision of health services and patient information and the role that health information technology can play in this regard, the present study is mainly concerned to investigate the effect of health information technology on time and cost saving.

Method

This is an applied study with a descriptive-analytical and cross-sectional method conducted on users of hospitals in remote areas of Iran in 2017, in which stratified random sampling method was used. Based on the Morgan formula, 256 subjects out of 720 Health Information System users were selected through stratified random sampling with proportional allocation. Data collection tool was a 24-question self-made questionnaire with 5-point Likert scale, designed using theoretical foundations. The first part of the questionnaire was measured the demographic information of the respondents. Out of 24 questions in the second part, 10 questions pertained to measuring the effect of health information technology on time saving and 14 questions pertained to measuring the effect of health information technology on cost saving. Considering that the Likert scale (very high, high, moderate, low, very low) was used for scoring, the range of scores varies from 1 to 5 in each phrase, therefore, all of the questions had a positive score, with the score 1 for very low and 5 for very high. Validity of the questionnaire was confirmed by health information management experts. In order to calculate the reliability, the questionnaire was distributed among 40 subjects and confirmed by Cronbach’s alpha coefficient 0.94. Data collection was conducted through in person referring to the workplace of the research population. If necessary, the research population was provided with explanations to the questionnaire items. Questionnaires were completed through self-administration at the same time and / or as a follow-up in the future. Data were analyzed by SPSS software version 21 and descriptive statistics (Frequency, standard deviation, mean,) and inferential statistics (Kruskal-Wallis, chi square tests) and the P-value was considered 0.01. Finally, the mean scores were classified, with the mean score of 1-3 in the low effect range, 4-4 medium effect and 4-5 high effect.

Findings

The majority of respondents aged 26 - 35, with a work experience of less than 5 years, with a Bachelor’s degree. According to the research findings, the effect of information technology on time and cost saving was positive in the perspective of the research population, with a mean score of 4.23. Subjects evaluated the effect of information technology on time saving with a mean score of 4.09 and on cost saving with a mean score of 4.37. Chi-square test results indicated a significant relationship between the effects of health information technology on time saving as well as cost saving (Table 1).

Table 1. The effects of health information technology on time saving as well as cost saving

<table>
<thead>
<tr>
<th>Variable</th>
<th>Chi2</th>
</tr>
</thead>
<tbody>
<tr>
<td>time saving</td>
<td>174.969</td>
</tr>
<tr>
<td>cost saving</td>
<td>169.813</td>
</tr>
</tbody>
</table>

The Kruskal-Wallis test was used to analyze the hypotheses of the relationship between the effect of health information technology on time and cost saving in terms of education level, age and work experience. Test results indicated no significant relationship between education level, age and work experience and people’s perspective on the effect of health information technology on time and cost saving (Tables 2 - 4).

Tables 2. The Kruskal-Wallis test on the effects of health information technology on time saving as well as cost saving by education level

<table>
<thead>
<tr>
<th>Education</th>
<th>Number</th>
<th>Chi2</th>
<th>Degree of Freedom</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Associate</td>
<td>118</td>
<td>1.864</td>
<td>2</td>
<td>.394</td>
</tr>
<tr>
<td>B.Sc</td>
<td>125</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.Sc</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>256</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Discussion**

The findings in the present study indicated that the amount of time saving of information technology is high in the perspective of users. This finding is consistent with the results of Dehghan and Ghorbani, Bertsche et al., Rollman et al., Lyerla et al., Walsh et al., Akbari-Nassaji et al., McMullin et al., and Nies et al.

To justify this issue, it can be stated that HIT plays a significant role in personal health management, health care delivery and public health. In these domains, widespread use of IT can improve the quality of health services, prevent medical errors, increase management efficiency, reduce paperwork and increase productivity of health services.

Concerning the effect of health information technology on cost saving, the findings also indicated that information technology has significantly led to cost saving. This finding is consistent with the results of Dehghan and Ghorbani, Bertsche et al., Rollman et al., Lyerla et al., Walsh et al., Akbari-Nassaji et al., McMullin et al., and Nies et al.

Investing in HIT has many benefits that may sometimes have measurable financial effects such as improving physician-patient relationships and increasing incentives for physicians and nurses, however, some of these benefits are simply measurable, including those that have the most benefits in the field of inpatient and outpatient care. These items include reducing the need for repeating tests and radiographs, reducing the cost and amount of medication, reducing patients’ length of stay, reducing the cost of documentation and reducing overtime and reducing nurses’ wasted time, increasing patient access to care, and reducing costs, decreasing the process time, increasing the variety of services provided, reducing the complaints from treatment personnel, and increasing the staff retention in their posts. In order to justify this point, it can be stated that according to the indicators of the US Health Care Management Information Center, there are fourteen areas of use in hospitals for information technology, so that using such applications are important in terms of affecting the quality of care, reducing costs and budgeting priorities.

Based on the results, there is no significant difference between the effects of HIT on time and cost saving and demographic information of users of hospitals in remote areas of Iran’s including education, age and work experience. Younger users reported that HIT had a greater effect on time and cost saving. Also, users who had less experience reported that HIT had a greater effect on time and cost saving. No similar research was found in this regard.

In general, the results of this study were consistent with the findings of Dehghan and Ghorbani, Bertsche et al., Rollman et al., Lyerla et al., Walsh et al., Akbari-Nassaji et al., McMullin et al., and Nies et al. The application of HIT not only enhances the status of clinical decision-making through the speed of...
and ease of information retrieval, but also affects other managerial and executive processes and other applied aspects of information in education and research which also leads to the effectiveness of the health system, which leads to the realization of the main goal of the health system, that is the promotion of community health. HIT plays a role in personal health management, health care delivery and public health. Of course, evidently achieving these benefits depends on the acceptance of technology by users, i.e. the positive attitude of users towards technology. The results of the study by Ayanlade et al., Kindratt et al. and Sebetci indicated that the strong understanding of staff and patients about the implementation and acceptance of HIT leads to achieving its benefits.

**Conclusion**

User satisfaction is one of the most important aspects of the information systems success. In addition, when using a new technology is supported by health professionals with respect to existing values and professional needs, they will not only have greater confidence in the use of IT, but also a higher degree of understanding and benefits of the system and will likely make better use of this technology, therefore it is recommended that senior managers and healthcare professionals pay more attention to the individual aspects of the organization, provide the training required prior to the implementation of information system, familiarize them with the benefits and capabilities of new technologies and also involve them in the decisions made to use such technologies. In the present study, it can be concluded that the positive view of the staffs in teaching hospitals of remote areas of Iran on the effect of HIT on time and cost saving indicates the availability of proper cultural groundwork for development of the information technology in university hospitals. Considering the growing global demand for health care information systems and competitive efforts in health care centers, the awareness of system managers and users on the effectivity of this technology on the qualitative and quantitative improvement of the health care services and developing a strategic HIT programs will guarantee the effectiveness of investing in this area with greater confidence.

**Acknowledgment:** Thereby the authors appreciate the cooperation and contribution of officials and personnel of the teaching hospitals of remote areas of Iran.

**References**


27. Sebetci Ö. Enhancing end-user satisfaction through technology compatibility: An assessment on health information system. Health Policy and Technology. 2018 Sep 1;7(3):265-74.
Iron Overload Complication in Thalassemia Patients

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Abstract

Thalassemia is an inherited disorder. All the types of thalassemia that require continuous blood transfusion, lead to increase of absorption of iron, which will lead to iron overload eventually. The precipitation of iron will affect many organs in the human body, and can be measured by S- ferritin.

In this study, patients with all kinds of thalassemia that require multiple blood transfusion, have been evaluated for the level of ferritin and iron overload as well as other complications such as liver diseases, heart diseases, bone disorders, and hormone disorders. One-hundred patients have been selected randomly with different ages to evaluate the serum ferritin, calcium level, liver enzymes, and Hb by use enzyme linked assay. This study confirms the use of chelation therapy to remove the iron overload.

Keywords: iron overload, thalassemia, GPT GOT, hormone disorder

Introduction

Hemoglobin, which is a red pigment that gives the RBCs their color, is presented around the RBCs. This pigment has the ability to transport loaded oxygen from the lung to other cells of body tissues and carries carbon dioxide from tissues toward lung. The globulin, which is the proteinaceous subunit of the hemoglobin, contains two kinds of chains; alpha and beta. These two chains are in association with the hem group that contains the iron atoms in form of Fe^2+. Despite the fact that the two chains are required for normal oxygen transportation, the alpha chain is expressed before birth, however, the beta chain does not and its expression is delayed for few months after birth. The synthesis of hemoglobin is controlled by specific genes in DNA. There are four genes control alpha chain expression whereas there are two genes control the beta chain expression.

The normal value of serum ferritin (a protein that stores iron in the body) is 160-180 mg/dl and the daily requirement is deferent from man and woman. In the adult man (19 years and older) the value is about 8mg/dl, whereas this value in the pregnant female may reach up to 27mg/dl. However, this value is only about 18mg/dl in non-pregnant women.

Many food sources are rich in iron especially animal products such as meat, liver, fish, and chicken. The non-hem sources such as plant iron.

We need to examine hemoglobin (HB test) in many cases such as the state of anemia (hemoglobin deficiency) and the iron overload which leads to hemochromatosis. Generally, the determination of the level of iron is carried out at the morning because during this period, the level of iron is in the maximum. Usually, the measurement of the level of iron, which represents the iron binding with transferrin, the total iron binding capacity, the ability of iron of binding with transferrin (TIBC) and the amount of iron. The mean the amount of iron that binds with transferrin is (400-250) mg/dl and transferrin saturation is (10%-50%) in men and (15%-50%) women ferritin. This gives the most indicators to the level of iron normally.

Heamatochromatosis means the accumulation of iron more than the normal value, which is genetic disorder, and may be resulted from the repetitive blood transfusion such as in thalassemia.

The symptoms and signs of Hematochromatosis are the change in the skin color into dark color, jaundice due to elevated bilirubin which gives yellowish of color of the skin, and painful joints that occurs due to iron precipitation in different organs of the body such that occurs in thalassemia.

Finally the iron overload (Haemotochromatosis), which occurs as a result of repeated transfusion of blood to the patient of thalassemia represents one huge...
problem to these patients because the human body does not have the ability to eliminate the excess iron, therefore, patients with thalassemia suffers from many disorders and disease and discoloration of patient such as liver disease, diabetes, heart abnormalities, disease Ferro protein disease, and decrease the secretion of hormones \(^{(4)}\)

Continuous blood transfusion to the patient with thalassemia the main reason of iron overload which precipitate in the deferent organ and tissue and cause many deferent problems, to prevent happen iron overload use long term of chelation treatment to prevent (indochrinopathies and cardiomyopathy) \(^{(5)}\)

**Material and Method**

About100 patients of thalassemia, who are all suffering from iron overload, have been selected randomly regardless of gender, age, and social background at the Center of Thalassemia in Kut province. by drawing venous blood from the patients, preserving their blood by cooling using the refrigerator not long to make the tests like s-calcium, s-ferritin and the other tests in table (2), and the instruments use in the tests, the electrophoresis and spectrophotometer, all the tests carried out in labs of kut center ofthalassemia treatment

**Statistical Analyses**

The data statistically analyzed by using spss-18 and sample t-test statistical program and the result of analyzing find the parameter report and the control and p-value for every parameter report p-value 0.05 or less consider significant.

**Findings**

The results showed that most of the patients were at the ages of youth and childhoods. Most of the sampled patients were from relative parents who were with different education levels; however, most of the parents were non-educated (Table 1). Most of the families were having one patient with the average value to the serum ferritin was about 4316 ng/ml. Deferoxamine, which is used as chelating factor to prevent overload of iron, is only used in patients who are less than 18 year of age. To diagnose thalassemic patients, electrophoresis method is used routinely in order to differentiate between the normal hemoglobin and defected hemoglobin. This test could differentiate the patients with thalassemia according to the particle size of the hemoglobin and electrical charge of these particles. All other parameters measure with colorimetric method which the concentration proportion with depth of color includes the serum Ca\(^{2+}\), serum ferritin, serum GOT, serum GPT, serum Urea, and serum Creatinin.

**Table 1. Classification of the thalassemia patients according to age, gender, and relativity.**

<table>
<thead>
<tr>
<th>Classes of age</th>
<th>frequency</th>
<th>Ra</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1</td>
<td>1</td>
<td>1%</td>
</tr>
<tr>
<td>1 - 5</td>
<td>15</td>
<td>15%</td>
</tr>
<tr>
<td>6 - 15</td>
<td>46</td>
<td>46%</td>
</tr>
<tr>
<td>16 - 25</td>
<td>26</td>
<td>26%</td>
</tr>
<tr>
<td>26 - 50</td>
<td>10</td>
<td>10%</td>
</tr>
<tr>
<td>50 &lt;</td>
<td>2</td>
<td>2%</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100%</td>
</tr>
<tr>
<td>Mean</td>
<td>10.75</td>
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</tr>
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<table>
<thead>
<tr>
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<th>frequency</th>
<th>Ra</th>
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</thead>
<tbody>
<tr>
<td>male</td>
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<td>54%</td>
</tr>
<tr>
<td>Female</td>
<td>46</td>
<td>46%</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Relationship</th>
<th>frequency</th>
<th>Ra</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non relatives</td>
<td>11</td>
<td>11%</td>
</tr>
<tr>
<td>relatives</td>
<td>89</td>
<td>89%</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100%</td>
</tr>
</tbody>
</table>
Table 1. Classification of the thalassemia patients according to age, gender, and relativity.

<table>
<thead>
<tr>
<th>Number of families with more than one patient</th>
<th>1</th>
<th>74</th>
<th>74%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
<td>20</td>
<td>20%</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>5%</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1</td>
<td>1%</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Types of disease</th>
<th>Major thalassemia</th>
<th>66</th>
<th>66%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>trait</td>
<td>23</td>
<td>23%</td>
</tr>
<tr>
<td></td>
<td>minor</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>sickle</td>
<td>3</td>
<td>3%</td>
</tr>
<tr>
<td></td>
<td>Thalassemia and sickle</td>
<td>2</td>
<td>2%</td>
</tr>
<tr>
<td></td>
<td>other</td>
<td>6</td>
<td>6%</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>

In this study 100 patients with thalassemia have been selected randomly during 2018. Their ages were from one month to 30 years. However, only few were at 51 years of age and most of them were less than 30 years. The number of male and female was nearly equal. The table also shows the presence or absence of more than one patient in the same family and the frequency of the complicating disease (Table 1). Most of patients were taking chelating factor Deferasirox.

Table 2. Differences in some serum parameters between patients with thalassemia and healthy looking people.

<table>
<thead>
<tr>
<th>variable</th>
<th>N</th>
<th>Patient gm/dl</th>
<th>df</th>
<th>control</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca mg/dl</td>
<td>100</td>
<td>8.9970±.80722</td>
<td></td>
<td>10</td>
<td>0.000 S</td>
</tr>
<tr>
<td>S ferritin ng/dl</td>
<td>100</td>
<td>4316.1800±311.32261</td>
<td></td>
<td>150±80</td>
<td>0.000 S</td>
</tr>
<tr>
<td>GOTI/L</td>
<td>100</td>
<td>38.9740±3.00802</td>
<td>99</td>
<td>28±4</td>
<td>0.000 S</td>
</tr>
<tr>
<td>GPIT/L</td>
<td>100</td>
<td>46.4200±5.00630</td>
<td>99</td>
<td>30±5</td>
<td>0.000 S</td>
</tr>
<tr>
<td>S-Bilirubin mg/dl</td>
<td>100</td>
<td>0.90.5±0.5</td>
<td></td>
<td>0.7±0.3</td>
<td>0.00s</td>
</tr>
<tr>
<td>B urea mg/dl</td>
<td>100</td>
<td>27.2500±1.59964</td>
<td>99</td>
<td>30</td>
<td>0.089 NS</td>
</tr>
<tr>
<td>S creatinin mg/dl</td>
<td>100</td>
<td>0.3630±.01186</td>
<td>99</td>
<td>0.4</td>
<td>0.070 NS</td>
</tr>
<tr>
<td>HB g/dl</td>
<td>100</td>
<td>8.92±0.8</td>
<td>99</td>
<td>13.±0.6</td>
<td>0.00 s</td>
</tr>
<tr>
<td>Iron ng/dl</td>
<td>100</td>
<td>250±70</td>
<td>99</td>
<td>75±15</td>
<td>0.00s</td>
</tr>
</tbody>
</table>

Table 2 shows the differences in value of some parameters between the patients' serum and the apparently healthy individuals. The value of the serum ferritin was highly elevated in patient than in control (4316± 311.22 ng/dl, whereas the normal 150± 80 ng/dl). No bilirubin was detected more than normal (no jaundice). In addition, enzymes of liver were also with high reading level than to the normal GOT and GPT as reported. The HB less than to the normal especial before transfusion HB=8.92±8 when the control equal13±0.6
so can see the deference and the anemia that the patient suffering as a result of decrease of HB.

**Discussion**

From the data parameters changing than to the normal as a result of elevated ferritin in blood, and this could be attributed to the precipitation of iron on deferent glands and organs. The free iron found in the blood is highly toxic to cells in the body. On the other hand, iron in serum bind with transferring, however, some of iron may present as non-binding iron especially when the concentration of iron exceeds the iron binding capacity of blood. There are a number of mechanisms to protect against the toxicity of free iron, which binds iron to various parts of the tissues. Ferritin appear in the blood although its role is unknown thalassemic patients need blood transfusion continually causing excess load of iron in human body. These patients not have any physiological method to remove excess load of iron. Non transferrin iron binds easily to translated with calcium canal to the liver (hepatocyte), heart (cardiac myocytes), and the other endocrine glands. The accumulation of iron in these organs leads to multiple complications. The most important complication of iron over load is the siderosis of heart, which may lead to heart failure, and considered as main reason of death. In addition, liver dysfunction could be observed for the same reason. Reports indicated that 25% of cardiac siderosis affect the patient of thalassemia in South East Asia and affecting 15 -20 % in Middle East (6).

From the data in (Table 1), we found that the level of calcium was less than normal in the patients because of the precipitation of iron on and in the parathyroid gland, which will reduce the secretion of parathyroid hormone. This hormone is the main factor that controls the level of calcium and phosphorus ion iron over load (7). This occurs in large number of patient of thalassemia as a result of increase absorption of iron in gastrointestinal tract and multiple transfusion of blood and inappropriate iron chelation therapy and this will lead to accumulation of iron in liver, endocrine glands, and heart eventually (8-9).

In our study, we found that the level of ferritin was 4316 gm/dl and this value is about eight fold higher than normal (Table 2), which lead to precipitate iron in liver and injury and fibrosis. Therefore, the levels of the transaminase enzymes s-GOT and GPT were highly elevated in comparison to the normal (s-GOT 39 IU/L and S-GPT 46 IU/L, respectively) (10,11,12).

Liver is the main organ to metabolism and for storage iron, hence, highly affected by the toxicity of iron. When iron accumulates in liver, it may cause lipid peroxidation may and may lead to hepatocyte necrosis and apoptosis. The end results for this pathway is hepatic fibrogenesis, which affects the collagen production and lead to fibrosis (13,14). As mentioned above, the complication of iron overload include heart disease (heart failure due to cardiac siderosis), chronic anemia, liver disease liver injury, liver fibroses, and as result viral hepatitis, hypogonadism, and bone disease. Our results indicated the absence of any effect of the iron overload on kidney and the parameters limited the health of renal function s-urea, and s-creatinin found in normal value as indicate in table (2).

**Conflict of Interest:** Non

**Source of Findings:** Self findings.

**Ethical Clearance:** Non

**References**


9. D.M. Styne, M.M. Grumbach Physiology and disorder of puberty


Relationship between Diet Quality and Obesity in Tikrit Secondary Schools Students

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1 Family and Community Medicine Department, College of Medicine, Tikrit University; 2 Basic Science, College of Dentistry, Tikrit University, Iraq

Abstract

This study to estimation the relation between diet quality and adiposity measures among secondary school students in Tikrit City. Patients and methods: cross-sectional descriptive epidemiological study was carried out to estimate the relation between diet quality and it’s association with anthropometric changes among 153 students (80 girls and 73 boys) in secondary school students in Tikrit city and also its association with demographic differences selected by means of a simple random sample. Results: In this study, a total of 153 secondary school students were surveyed. The majority were females (52.29%) and most of them (41.25%) were between 16-18 years old. Most of them (55.56%) had a BMI range from 18.5-25 and their waist-to-height ratio (< 0.5) were (67.32%). (32.03%) of students had a family history of obesity while (67.97%) had a negative family history. Conclusions: Most of the students have a normal BMI (55.56%) , so the frequency of overweight was more common in female gender (60%), while in male (50.68%). And (32.03%) of students had a positive family history of obesity while (67.97%) had negative family history, From students who have a positive family history, (32.66%) had an overweight BMI (25-30) and (44.9%) waist-to-height ratio which considered as indication of central obesity.

Keywords: diet quality ; adiposity measures ; secondary school students ; Tikrit city

Introduction

In adolescence and childhood , it was suggested that food should have a lasting impact on the child’s development in various aspects of life also on Chronic disease as the obesity (1) . Good nutrition with healthy diet during childhood and adolescence is essential for growth, development, health and well-being (2). Unhealthy diet is considered as a behavioral risk factor for hypertension and other cardiovascular disease (3). Obesity increased in the societies due to poor eating habits like increase consumption of sweetened beverages, energy-dense foods and change in the eating behaviour to Consumption of refined grains; fats added; sugars added; snacks; Drinks; eat away from home (4). Obesity is often defined as a state of abnormal or excessive fat accumulation in adipose tissue, to the extent that health may be impaired (5) and obesity is measured in various ways such as body mass index (BMI); percentage of body fat measurements; Waist-to-hip ratio; waist circumference; and skinfold (5).

High rate of overweight and obesity in many countries has been described as a global pandemic (6-8). In the previous, the obesity is a problem in only high income countries , but now obesity and overweight become increased in both low- and middle-income countries, especially in urban region (9).

In 2016, more than 1.9 billion adults, 18 years and more than , were overweight from 650 million were obese (10), also in in 2016 more than 41 million children with age less than 5 years suffering from overweight or obese (11). Also in 2016, more than 340 million children and adolescents with aged range from 5-19 were suffering from overweight or obese (11). So prevalence of overweight and obesity among children and adolescents in age range 5-19 years were increased from 4% in 1975 to 18% in 2016, overweight in boy (19%) more than girls (18%) (11). Nearly 1 in 5 children and adolescents are overweight or obese (11).

Diet quality also associated with mental health problems such as depression (12). Eat perfect food is associated with an increase in life expectancy, a
significant reduction in the risk of chronic disease throughout life, and improved gene expression\(^{(13)}\).

In one study found good diet quality associated with 12-28\% reduced risk of all causes, cardiovascular disease and cancer mortality in men and women\(^{(14)}\).

Good diet quality should associate with good water as Good hydration is vital for good health and well-being\(^{(14)}\), and physical activity as it reduces the risk of chronic disease and obesity\(^{(14)}\).

Physical inactivity was associated with higher risk of diabetic (type-2), regardless of BMI ; Gender ; age and ethnicity \(^{(15)}\). Individuals with mild, moderate or active physical activity were at lower risk of CVD deaths, regardless of their metabolic risk factors \(^{(16)}\), therefore the aim of current study to estimate how diet quality of meals can affect anthropometric measures ; identify amount of water intake and its association with anthropometric measures ; identify students with a family history of obesity and its association with anthropometric measures; determine daily activities of students, including the frequency and duration; and measure frequency of obesity among secondary school students in Tikrit city.

Patients and Methods

Cross-sectional study was used to assess the diet quality of secondary school students in Tikrit city and its association with anthropometric measures (BMI and waist-to-height ratio). The duration of the whole study was almost four months, the data was collected from the 1\(^{st}\) of November to the 1\(^{st}\) of January. The data was analyzed and the report was written from 1\(^{st}\) of January to the 25\(^{th}\) of March. The data was collected by simple random sample and the number of samples are 153 cases of both genders from secondary school students.

The data was collected by the members of our study group from the following secondary schools (Ibn-almotam ,Al-Khansaa and Al-Mustansrya). A suitable questionnaire was designed in Arabic language and contained (name, age ,class ,gender ,types of foods eaten regularly by students ,water intake ,activity ,family history of obesity) to achieve the objectives. The questionnaire directed by interviewer.

The weight is measures for each student participant in the study. It is measures without shoes. The height of students is measures without shoes by using measuring tape of height 2 meters. The individual should stand on a flat surface, the head upward and the head, back and heel are against the wall.

The BMI is calculated as weight (kilograms) / height (meters)squared. The height was taken (to the nearest 0.1centimeter) and weight (to the nearest 0.1kilogram). Waist: height ratio will calculated as waist circumference / height and the measures was taken to the nearest 0.1centimeter. We explained the purpose and aim of the study and only those who agreed to participate in the study and we also focus on the privacy of the information that taken from the students.

Finding

A total of 153 secondary school students were surveyed. The majority were females (52.29\%) and most of them (41.25\%) were between 16-18 years old. Most of them (55.56\%) had a BMI range from (18.5-25) and their waist-to-height ratioas(\(< 0.5\)) were (67.32\%) so (32.03\%) of secondary school students had a family history of obesity while (67.97\%) had a negative family history.

Table (1) Distribution of secondary school students according to demographic characteristics of the study sample

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Males</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
</tr>
<tr>
<td>Gender</td>
<td>73</td>
<td>47.71</td>
<td>80</td>
</tr>
<tr>
<td>Age( years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12-14</td>
<td>19</td>
<td>26.03</td>
<td>16</td>
</tr>
</tbody>
</table>
Cont... Table (1) Distribution of secondary school students according to demographic characteristics of the study sample

<table>
<thead>
<tr>
<th>Age Group</th>
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<th>Percent Female</th>
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<tr>
<td>&gt;14-16</td>
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<td>21.92</td>
<td>21</td>
<td>37</td>
<td>24.18</td>
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<td>&gt;16-18</td>
<td>30</td>
<td>41.1</td>
<td>33</td>
<td>63</td>
<td>41.18</td>
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<tr>
<td>&gt;18</td>
<td>8</td>
<td>10.96</td>
<td>10</td>
<td>18</td>
<td>11.76</td>
</tr>
</tbody>
</table>

Family history of obesity

<table>
<thead>
<tr>
<th>Category</th>
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<th>Percent Female</th>
<th>Total</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>23</td>
<td>31.51</td>
<td>26</td>
<td>49</td>
<td>32.03</td>
</tr>
<tr>
<td>NO</td>
<td>50</td>
<td>68.49</td>
<td>54</td>
<td>104</td>
<td>67.97</td>
</tr>
</tbody>
</table>

Figure (1): - percent of secondary school students who eat these important sources of carbohydrate in a regular manner weekly.

The figure shows bread as the most common source of carbohydrate in diet among male students 59/73 (80.82%), and also female 63/80 (78.75%).

Also The figure shows that second most common source of carbohydrate in diet among male students 57/73 (78.08%) was rice, and also female 58/80 (76.25%).

Figure (2): - percent of secondary school students who eat these important sources of protein in regular manner weekly.
The figure shows poultry as the most common source of protein in diet among female students 71/80 (88.75%), and also male 63/73 (86.3%).

Also, the figure shows that the second most common source of protein in diet among male students 48/73 (65.75%) was egg, and also female 41/80 (51.25%).

![Figure (3): percent of secondary school students who eat these important sources of saturated fat in regular manner weekly.](image)

The figure shows milk and dairy product as the most common source of saturated fat in diet among male students 42/73 (57.53%), and also female 38 (47.5%).

Also, the figure shows that red meat comes behind milk and dairy product as a second source of saturated fat in diet among male students 32/73 (43.84%), and also female 30/80 (37.5%).

![Figure (4): percent of secondary school students who eat these important sources of unsaturated fat in regular manner weekly.](image)

The figure shows olive as the most common source of unsaturated fat in diet among female students 42/80 (52.5%), and also male 35/73 (47.95%).
Also The figure shows that the nut comes behind olive as a second source of unsaturated fat in diet among male students 26/73 (35.62%), and also female 24/80 (30%).

### Table (2): - body mass index (BMI) score of the secondary school students involved in the study according to the gender.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>BMI</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number</td>
<td>Percents</td>
<td>Number</td>
</tr>
<tr>
<td>18.5&gt;</td>
<td></td>
<td>14</td>
<td>19.18</td>
<td>8</td>
</tr>
<tr>
<td>18.5-25</td>
<td></td>
<td>37</td>
<td>50.68</td>
<td>48</td>
</tr>
<tr>
<td>25-30&lt;</td>
<td></td>
<td>15</td>
<td>20.55</td>
<td>16</td>
</tr>
<tr>
<td>&lt;30</td>
<td></td>
<td>7</td>
<td>9.59</td>
<td>8</td>
</tr>
</tbody>
</table>

This table shows that Most of the secondary school students 85/153(55.56%) had a BMI range from 18.5-25, were 48/80(60%) from female and 37/73(50.68%) from male within this BMI range.

### Discussion

The majority of secondary school students with normal BMI (55.56%) and their waist-to-height ratio (<0.5) were (67.32%) and this is matching a result from a research which was done 6 years ago in United states of America that found two thirds of samples had a normal BMI and waist-to-height ratio was 0.49. (17)

According to this study, the most common age group was between 16-18 years which was (41.18%), and this agree with research was conducted in United States of America in 2012 which show that the most affected age group were between 12 18 years old (42.3%). (17) According to this study, the most common source of protein was poultry (88.75%) among females and in males (86.3%) and this is agree with another study done in United kingdom in 2012. (16) According to this study, the most common source of carbohydrate was bread (80.82%) among male and among females (78.75%) while in another study done in Norfolk, United Kingdom at 2012 found that fruit and vegetables were more common but this study not mention the percentage (16)

In this study the percentage of family history of obesity in students was (32.03%) and this is disagree with another study in Kirkuk city which reveal that (59.2%) of them has a family history of obesity (16). Also this matches with another study occurred in Kirkuk city before 5 years (61.5%) was the percent of secondary school students with normal BMI, this study indicated that female students (60%) more obese than male students (50.68%) and this disagree with a study in Erbil that reveal male students (18).

### Conclusion

- Most of the students have a normal BMI (55.56%).
- The frequency of overweight was more common in female gender (60%), while in male (50.68%).
- The frequency of obesity was more common in female gender (10%), while in male (9.59%).
- Most common age group involved in the study were between 16-18 and account for (41.18%).
- (32.03%) of students had a positive family history of obesity while (67.97%) had negative family history.
- From students who have a positive family history, (32.66%) had an overweight BMI (25-30) and (44.9%)waist-to-height ratio which considered as indication of central obesity.
• Regarding carbohydrates, the most common source taken by students is bread (79.74%).

• Regarding protein, the most common source taken by students is poultry (87.58%).

Conflict of Interest: None.

Source of Funding: Self funding.

Ethical Clearance: Taken from student and college.

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Relation of Epstein Barr virus with interleukin-10
Level among men with Prostate Cancer in Ramadi City

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Abstract
The study aimed at evaluating the relation of Epstein Barr virus (EBV) with level of interleukin-10 (IL-10) in men with prostate cancer (PC). The study was carried out in Ramadi city from 12th of January to 12th of September 2018, and included a total of 95 women with breast cancer who admitted to oncology clinic of Ramadi Teaching Hospital whose ages were between 40-80 years. Patients were investigated for detection of EBV by using Real Time PCR and interleukin-10 (IL-10) by ELISA technique. The rate of prostate cancer men with EBV was (16.84%). The highest rate of prostate cancer men was within the age group 50-59 years with no significant relation between prostate cancer and age. The study showed that the highest means of IL-10 level (19.43 pg/ml) were found in prostate cancer men without EBV. There was significant difference (P value < 0.05) between EBV infection with IL-10. The highest rate of men with prostate cancer was from urban areas.

Keywords: Prostate cancer, EBV, IL-10, Ramadi.

Introduction
Prostate Cancer (PC) is the second most common cancer among men in western populations, and despite its high mortality, its etiology remains unknown. Inflammatory processes are related to the etiology of various types of tumors, and prostate inflammation, in particular, has been associated with prostate cancer carcinogenesis and progression (1).

Epstein- Barr Virus (EBV) has been linked to the development of variety of human malignancies including prostate tissues that range from benign prostatic hyperplasia (BPH) to prostatic adenocarcinoma (PAC). Somatic point mutations in Rb gene have been detected in prostate cancer and are involved in progression steps of prostate carcinogenesis (2).

Interleukin-10 (IL-10) is a key regulator of immune responses described as cytokine synthesis inhibitor, immune suppressive and anti-angiogenic factor produced by Thelper2 (Th2) cells and inhibits Thelper1 (Th1) cells by inhibiting pro-inflammatory cytokines. In addition, IL-10 can inhibit monocyte/macrophage functions including monokine synthesis, nitric oxide production, and major histocompatibility complex (MHC) class II and CD80/CD86 co-stimulatory expression. In vitro and in vivo studies revealed pleotropic activities of IL-10 on B and T cells and, taken together, that a critical function of IL-10 is to suppress multiple immune responses through individual actions on T and B cells, antigen presenting cells and other cell types, and skew the immune response from Th1 to Th2. In malignancy, IL-10 might promote tumor development, by acting to suppress anti- tumor immune responses(3).

Material and Method
Across a sectional study was carried out in Ramadi city from 12th of January to 12th of September 2018, and included 95 men with prostate cancer whose ages were between 40-80 years old. These patients admitted to oncology clinic of Ramadi Teaching Hospital.

Blood samples were taken from prostate cancer men. Samples were examined by immunological methods, enzyme linked immuno sorbent assay (ELISA) for detection interleukin-10(IL-10) and molecular technique (real time PCR) which included DNA amplification of Epstein Barr virus based on the specific primers.

Seven and half ml of blood was collected by vein puncture using vacutainer tubes from each patient
enrolled in this study. Blood samples were divided into two sterile test tubes, in one of them 2.5 ml of blood was put in test tube containing anticoagulant ethylene diamine tetra acetic acid(EDTA) and used for DNA extraction ofEBV. The second part of sample (5ml) was placed in plain tubes left for 30 minutes at 37 °C then was centrifuged at 3000 round per minute(rpm) for 15 minutes then the clot was removed and the remain re-centrifuged at 3000 rpm for 10 min and the obtained sera were then aspirated using automatic micropipette and transferred into two clean test tubes, for serological tests. Label was fixed on each test tube which then stored in deep freeze at -20°C for late serological testing for determination the level of IL-10 by using ELISA technique.

For DNA extraction, kit was purchased from Gene Aid(USA) company for molecular detection of EBV by Real Time PCR using Anatolia Gene Works(Turkey). Detection of IL-10 was done by using ELISA kit Elabscience(China), which depends on the Sandwich-ELISA principle.

**Statistical Analysis**

Computerized statistically analysis was performed using T-Test probability. The $P$ value $>0.05$ was considered statistically significant, and for result which its $P$ value was less than 0.01 was considered highly significant, while for those which its $P$ value greater than 0.05, was considered statistically non-significant.

**Findings**

A total of 95 prostate cancer men, their age ranged between 20-89 years old, were investigated for detection of EBV by using real time PCR and estimation the level of IL-10. The present study revealed that EBV was detected in 16.84% of men with prostate cancer, as shown in Table 1.

<table>
<thead>
<tr>
<th>EBV</th>
<th>Prostate Cancer Men</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Positive</td>
<td>16</td>
</tr>
<tr>
<td>Negative</td>
<td>79</td>
</tr>
<tr>
<td>Total</td>
<td>95</td>
</tr>
</tbody>
</table>

The current study showed that the highest rate of EBV infection (7.36 %) was found in men of prostate cancer within the age group 50-59 years. Table 2.

<table>
<thead>
<tr>
<th>Age Group (Years)</th>
<th>No. of Men with Prostate Cancer</th>
<th>EBV Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>20-29 (No:0)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>30-39 (No:3)</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>40-49 (No:15)</td>
<td>15</td>
<td>1</td>
</tr>
</tbody>
</table>
The present study revealed that there was a negative correlation between EBV infection with age of men with prostate cancer, but the relation was statistically non-significant. (R value: -0.09) (Figure 1).

The current study showed that the highest rate of men with prostate cancer and EBV- infection was from urban areas (68.42%), as shown in Table 3.

Cont. Table 2: Distribution of EBV infection according to age of men with prostate cancer.

<table>
<thead>
<tr>
<th>Age Group (Years)</th>
<th>EBV Positive Cases</th>
<th>Total Cases</th>
<th>EBV Infection Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>50-59 (No:38)</td>
<td>38</td>
<td>7</td>
<td>7.37</td>
</tr>
<tr>
<td>60-69 (No:27)</td>
<td>27</td>
<td>5</td>
<td>5.26</td>
</tr>
<tr>
<td>70-79 (No:6)</td>
<td>6</td>
<td>3</td>
<td>3.16</td>
</tr>
<tr>
<td>80-89 (No:6)</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total (No:95)</td>
<td>16</td>
<td>16.84</td>
<td></td>
</tr>
</tbody>
</table>
Table 3: Distribution of men with prostate cancer according to residence.

<table>
<thead>
<tr>
<th>No. of Men with Prostate Cancer</th>
<th>Residence</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rural</td>
<td>%</td>
<td>Urban</td>
</tr>
<tr>
<td>No.</td>
<td>30</td>
<td>31.58</td>
<td>65</td>
</tr>
</tbody>
</table>

The highest rate of men with prostate cancer who were infected with EBV were from urban areas (14.73 %), as shown in Table 4.

Table 4: Distribution of EBV infection according to residence of men with prostate cancer.

<table>
<thead>
<tr>
<th>Residence</th>
<th>No. of Patients</th>
<th>EBV Infection</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Rural</td>
<td>30</td>
<td>2.11</td>
<td>2</td>
</tr>
<tr>
<td>Urban</td>
<td>65</td>
<td>14.73</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>95</td>
<td>16.84</td>
<td>16</td>
</tr>
</tbody>
</table>

Relation of EBV infection with IL-10 among prostate cancer men.

Table 5 shows that the means of IL-10 level was higher in prostate cancer men without EBV infection (19.43), highly significant P value <0.05 between EBV and IL-10.

Table 5: Relation of EBV infection with IL-10 among prostate cancer men.

<table>
<thead>
<tr>
<th>EBV Infection</th>
<th>No. of Men with Prostate Cancer</th>
<th>IL-10 Mean</th>
<th>SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ve</td>
<td>16</td>
<td>13.50</td>
<td>±3.39</td>
<td>0.00</td>
</tr>
<tr>
<td>-ve</td>
<td>79</td>
<td>19.43</td>
<td>±10.35</td>
<td></td>
</tr>
</tbody>
</table>

The present study revealed that there was a positive correlation between EBV infection with IL-10 among men with prostate cancer but the difference between them was statistically non-significant (R value: 0.198).

Prostate cancer is the second most frequently diagnosed neoplasm in men and the fifth cause of cancer death worldwide (4). Viruses are etiologic factors in the development of several types of human tumors. At least 15–20% of all human tumors worldwide have a viral cause (5). The present study revealed that 16.84% of men with prostate cancer had EBV infection. Several studies confirmed the presence of EBV in prostate tissues. In Sweden, EBV was present in 8.8% of benign and malignant prostate tissues (6), and in the United States, EBV was present in 8% of all normal, benign, and malignant prostate tissues (7). In another study, approximately 37% of prostate cancer patients had EBV infection (8).

The current study showed that the highest rate of EBV infections (7.36%) was found in those within the age group 50–59 years. The reason for EBV to exert its oncogenic influences in a particular patients is unknown but is probably associated with co-factors. The findings in the research by have supported hypothesis that the prostate is a habitat for multiple viral and other infectious agents, some of which have oncogenic potential (9).

The current study showed that no significant difference was found between EBV infection and residence in men with prostate cancer P value > 0.05. The study of urban–rural differences in prostate cancer in Australia showed that the higher rates of prostate specific antigen (PSA) testing particularly aggressive prostate cancer in regional and rural Australia. Other studies have found urban–rural differences in the management of other cancers (10).

The highest mean of IL-10 level was in men with prostate cancer without EBV infection (19.43%). There was significant difference P value < 0.05 between EBV with IL-10, and there was a positive correlation between EBV infection and IL-10. Several studies have indicated that IL-10 has both pro- and anti-tumoral effects. IL-10 inhibits NF-κB signaling; therefore, it can down regulate pro-inflammatory cytokine expression (11). Interleukin-10 is immunosuppressive and anti-inflammatory. Interleukin-10 inhibits NF-κB activation through ill-defined mechanisms, and consequently inhibits the production of pro-inflammatory cytokines,
including TNF-α, IL-6, and IL-12 \(^{(12,13,14,15)}\).

**Conclusions**

The present study revealed that 16.84% of men with prostate cancer had infection with EBV. The relation between EBV infection and age was statistically non-significant. The highest rate of men with prostate cancer who were infected with EBV was from urban areas. The highest mean of IL-10 level was detected in men without EBV infection.

**Conflict of Interest**: non

**Source of Findings**: self findings.

**Ethical Clearance**: non

**References**


A Histological Study of the Renal Corpuscle Nephron Distribution in Iraqi Camels (Camelus dromedaries)

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2Department of Anatomy and Histology / College of Veterinary medicine- University of Baghdad, Iraq

Abstract

Aims: The present histological study was carried out to map the renal corpuscle nephron distribution and their histological properties in Iraqi camels(Camelus dromedaries). Materials and methods: Both kidneys from each of 10 (5 males and 5 females) adult camels were collected as soon as possible after the camels were sacrificed for human consumption purposes in a slaughterhouse. Following the preparation of tissues(cortex and medulla), the sections were slide-stained using alum hematoxylin and eosin (H&E) stain and Periodic Acid Schiff (PAS) stain. The renal corpuscle diameters (numbers and percentages) in the subcapsular area, the cortical area, and the juxtamedullary area were measured using an ocular micrometer. Results: The findings demonstrated that the renal capsule in camels is thick. The numbers of the renal corpuscles were low in numbers in the subcapsular region which keep increasing towards the midcortical and the Juxtamedullary regions. Moreover, the camel kidney has high numbers of long-loops of Henle nephrons and low numbers of short-loop nephrons. No significant (p<0.05) differences in the renal corpuscle diameters were noticed between midcorticaland juxtamedullaryareas. However, a significant (p<0.05) decrease in diameters of the renal corpuscles was seen in the subcapsular region. In addition, two layers consist the renal corpuscles with a tuft of capillaries. The proximal convoluted tubules (PCTs) showed wide lumens with the presence of cuboidal epithelial cells (CuECs) and spherical nuclei. The distal convoluted tubules (DCTs) displayed the presence of CuECs (smaller and lighter than those in the PCTs with apical spherical nuclei (ASN). Large lumens and simple columnar epithelial cells (CoECs). Furthermore, PAS staining showed high positive results in the basement membrane of the renal corpuscles. Conclusion: The current histological study provides insight about the renal corpuscle nephron distribution and their histological properties in Iraqi camels(Camelus dromedaries).

Keywords: Camels (Camelus dromedaries), nephron, renal corpuscles.

Introduction

In Iraq, camels are considered as one of the main economic resources that provide the country with various forms of human needs such as meat and milk consumption and, in some areas, as a transportation method. The major cleaning-out organs in the camel body are the bean-like shaped kidneys. The kidneys provide the body with very important roles such as getting rid of the end-products of the body metabolic processes. The kidneys also enhance body balance of fluids, electrolytes, and acid versus base ratios by changing the solute concentration and water volume in urine(1).

Moreover, kidneys in camels have specific anatomical characteristics that allow for hypertonic-based urine production via the camel-specialized loop of Henle-collecting tubule distance that promotes for maximum reabsorption of water from urine(2;3;4;5). Interestingly in camels, the cortical layer can reach up to the half of the kidney size with a ratio of 4:1 of medulla to cortex of thickness(6).

Renal insufficiency, as shown by various researchers, could be due to pre-renal, renal, and post-renal causes, however, nephritis and glomerulonephritis are among rare disease conditions that affect camels with low incidence rates which had only been discovered in slaughtered camels(7;8).

The present histological study was carried out to map the renal corpuscle nephron distribution
and their histological properties in Iraqi camels (*Camelus dromedaries*). Understanding those anatomical properties in camels may enhance better knowledge for developing new medicines and techniques to treat various kidney diseases in camels.

**Materials and Method**

**Animals and samples**

Both kidneys from each of 10 (5 males and 5 females) adult camels were collected as soon as possible (within 20 mins) after the camels were sacrificed for human consumption purposes in slaughterhouses distributed in various regions of middle of Iraq. The kidneys were rapidly rinsed and placed in 10% buffered neutralized formalin solution for 48 hrs.

**Tissue and tissue section preparation**

Following the preparation of tissues (cortex and medulla) that were dehydrated, cleared, and embedded in paraffin wax and cut-sectioned for pieces measured 5 to 7 µm by employing a rotary microtome, the sections were slide-stained using alum hematoxylin and eosin (H&E) stain and Periodic Acid Schiff (PAS) stain. The renal corpuscle diameters (numbers and percentages) in the subcapsular area, the cortical area, and the juxtamedullary area were measured using an ocular micrometer.

**Statistical Analysis**

The renal corpuscle diameters (numbers and percentages) in the subcapsular area, the cortical area, and the juxtamedullary area were measured using an ocular micrometer. Those data were analyzed using an F-test. The null hypothetical problem was refused if $p$ was less or equal to 5%. The finding values were processed using SPSS software.

**Findings**

The findings demonstrated that the renal capsule in camels is thick. The numbers of the renal corpuscles were low in the subscapular region which keep increasing towards the midcortical and the juxtamedullary regions. Moreover, the camel kidney has high numbers of long-loops of Henle nephrons and low numbers of short-loop nephrons. No significant ($p > 0.05$) differences in the renal corpuscle diameters were noticed between midcortical and juxtamedullary areas. However, a significant ($p < 0.05$) decrease in diameters of the renal corpuscles was seen in the subcapsular region, table 1.

**Table 1: Distribution and diameters of renal corpuscle regions in camel kidneys.**

<table>
<thead>
<tr>
<th>Renal corpuscle regions</th>
<th>Mean±SE (%)</th>
<th>Diameter (µm)/Mean±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-capsular</td>
<td>27.5 ± 0.83 a</td>
<td>112.22 ± 3.354 c</td>
</tr>
<tr>
<td>Mid-cortical</td>
<td>41.9 ± 1.13 b</td>
<td>125.38 ± 2.675 d</td>
</tr>
<tr>
<td>Juxtaglomerular</td>
<td>31.3 ± 0.107 c</td>
<td>124.23 ± 2.654 d</td>
</tr>
</tbody>
</table>

Different letters refer to significant ($p \leq 0.05$) differences.

In addition, two layers consist the renal corpuscles with a tuft of capillaries. The proximal convoluted tubules (PCTs) showed wide lumens with the presence of cuboidal epithelial cells (CuECs) and spherical nuclei. The distal convoluted tubules (DCTs) displayed the presence of CuECs (smaller and lighter than those in the PCTs with apical spherical nuclei (ASN)). Large lumens and simple columnar epithelial cells (CoECs). Furthermore, PAS staining showed high positive results in the basement membrane of the renal corpuscles, figure 1A, B, C, and D.
Discussion

The numbers of correctly functioning renal nephrons decide the proper action of a kidney; however, kidneys may face ineffectiveness in their work if there is insufficient renal blood supply, deficient in the glomerular filtration, and defectiveness in the reabsorption of tubules. The first factor can be pre-renal initiated due to some circulatory emergency conditions such as dehydration, hemorrhage, and shock in responses to vasomotor control. While the other two factors can be induced as results to improper functions of the kidney itself (11).

The current work was focused on finding the actual histological distribution of the renal corpuscles in the camel kidney which might help better understanding the functions of the kidney nephrons.

The findings demonstrated that the renal capsule in camels is thick. Interestingly in camels, the cortex can reach up to 50% of the kidney size, and this shows the huge length of the loops of Henle and vasa recta, also discovered in the current study, which adds more evidence that camel kidneys are able to concentrate their urine as part of their fulfillment of water preservation (12).

A significant decrease in diameters of the renal corpuscles was seen in the subcapsular region. In addition, two layers consist the renal corpuscles with a tuft of capillaries. The PCTs showed wide lumens with the presence of CuECs and spherical nuclei. This piece of result agrees with the previously identified facts that renal corpuscles and glomeruli in camel kidneys are larger than those recognized in kidneys of other animals, and this is supported by the approved ideas that camels suffering dehydration have 73% reduction of the reabsorption of sodium from tubular lumens resulting in concentrating the camel urine and better preserving water (13;14). Furthermore, PAS staining showed high positive results in the basement membrane of the renal corpuscles. This indicates highly normal glomeruli of the camel kidneys with presence of glycoprotein, glycolipid, and mucin structural components (15).

Conclusion

The current histological study provides insight about the renal corpuscle nephron distribution and their histological properties in Iraqi camels (Camelus dromedaries).
Conflict of Interest: Non

Source of Findings: Self Findings.

Ethical Clearance: Non

References


Correlated between Sera Levels of Interleukins (IL-6, IL-17 and IL-23) with Virulence Genes Detected in Carbapenem-Resistant E. coli

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¹Biology department, College of Science, Al-Mustansiriya University, ²Medical laboratory techniques Department, College of Health & Medical techniques, Middle Technical University

Abstract

Current study demonstrated that 14.58% of isolated E. coli which positive for each of Omp-A, IMP-A, NDM-A and Fim-H genes were resistant to carbapenem and the study showed that 30.51% of E. coli isolates with KPC were resistant to carbapenem while other E. coli isolates with negative all genes were resistant to carbapenem, so the percentage as 72.92% of isolated E. coli were positive for each of Omp – A, IMP – A, NDM – A and Fim – H genes were occurred in females compared with 27.02% occurred in males and 40% of isolated E. coli with positive for KPC gene were in females with UTI. as well as 10, 50% of isolated E. coli were positive for each of Omp - A, IMP - A, NDM - A and Fim - H genes were occurred in the age group 15-24 year and no isolate were form patients above 54 year.

the highest mean levels IL-6 and IL-17 was recorded in UTI patients infected with E. coli positive for each of Omp-A, IMP-A, NDM-A and Fim-H comparing with patients infected with E. coli negative to these genes. Conclusion Carbapenem-resistant E. coli correlated with founded each of genes ( Omp-A, IMP-A, NDM-A, and Fim-H ) , whilst highest correlated between Carbapenem-resistant E. coli with KPC.so sera levels of both interleukins (IL-6 and IL-17) were recorded in UTI patients infected with E. coli positive for each genes ( Omp-A, IMP-A, NDM-A and Fim-H ) comparing with patients infected with E. coli negative to these genes.

Keywords: sera levels of interleukins (IL-6, IL-17 and IL-23); virulence genes; carbapenem-resistant E. coli

Introduction

E. coli belongs to the Enterobacteriaceae family and is present in the natural microbiota of humans and other homoeothermic animals. The E. coli associated with UTIs are denoted uropathogenic E. coli(UPEC) (¹), With the widespread use of antibiotics, the carbapenem-resistant strains have become a serious public health issue in the worldwide and are usually resistant to almost antibiotics (²). Carbapenem-resistant Enterobacteriaceae (CRE), specially included three species of the Enterobacteriaceae family, the Klebsiella, Enterobacter and Escherichia coli have developed resistance to a group of antibiotics called “Carbapenems”, which are often used as the last line of treatment when other antibiotics are not effective in treating infections caused by them (³). Carbapenem-resistant E. coli strains is a main risk for global public health, but little is known of carbapenemase producing E. coli in Iraq.

E. coli strains have some gene regions responsible for virulence factors which may encode adhesins, toxins, siderophores and haemolysin. Outer membrane protein A (OmpA) is a major protein in the Escherichia coli outer membrane, it is the abundant outer membrane proteins (OMPs), with typically 100,000 copies per cell (⁴).

Type I fimbriae, coded by plasmid-mediated fimA gene and commonly found in these strains from lower urinary system infections, enable E. coli to adhere to human ureteral mucosa epithelial cells (⁵). Afimbrial adhesin encoded by plasmid- or chromosomemediated afa and S fimbriae encoded by plasmid sfa gene regions are commonly found in urinary system infection originated in isolates as well as sepsis and meningitis (⁶).
The most important virulence factors for urinary tract infections are fimbriae. It was reported that no important difference in presence frequency of type I fimbria between low and high virulence isolates in the urinary tract (7) (Plos et al., 1991).*FimA*, associated with ancillary proteins *FimF, FimG*, and the adhesin protein *FimH*, encoded by the *fim* gene cluster (8). This type of fimbria is common among Enterobacteriaceae, also several variants have been strongly associated with UPEC (9). Their role in infection is unclear, although it has been suggested that they may be involved in the initial stages of colonizing in the upper respiratory tract (9;10).

Interleukin (IL)-6 is a pro-inflammatory cytokine, the concentration of which increases in the early stage of bacterial infection. Interleukin-6 has regenerative activities, which, when absent, aggravated the development of the inflammatory process.

So IL-23 is believed to be important in the expansion and survival of these IL-17-producing cells (naïve T cells) (11). In addition, an intact IL-23-IL-17 axis seems to be essential for host protection against infections, as well as in the pathogenesis of certain autoimmune diseases (12). Aim of current study was evaluate the possible correlations between carbapenem-resistant *Escherichia coli* and frequency of virulence gene in UTIs infections, and correlated between carbapenem-resistant *E. coli* and level of IL-6; IL-17 & IL-23.

**Materials and Methods**

**Primers Used in Current Study:** All the primers used in this study listed in table (1).

### Table (1): All Primers used in this study

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence of forward and reverse Primer(5’ - 3’)</th>
<th>Product bp</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Fim</em> (h)</td>
<td><strong>F</strong> TGC AGA ACG GAT AAG CCG TGG  <strong>R</strong> GCA GTC ACC TGC CCT CCG GTA</td>
<td>508</td>
<td></td>
</tr>
<tr>
<td><strong>(5’ - 3’)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OmpA</td>
<td><strong>F</strong> ACCCTGGTTGTAAGCGTCAG  <strong>R</strong> GTAAAAACGACGACCGGCG</td>
<td>419</td>
<td>Alpha DNA Co.(Canada)</td>
</tr>
<tr>
<td>KPC</td>
<td><strong>F</strong> CGTCATAGCTGCTGCTTTG  <strong>R</strong> CTTGTCATCTTGTGTTAGGCG</td>
<td>798</td>
<td></td>
</tr>
<tr>
<td>NDM-1</td>
<td><strong>F</strong> ACCCTGGTTGTAAGCGTCAG  <strong>R</strong> GTAAAAACGACGACCGGCG</td>
<td>621</td>
<td></td>
</tr>
<tr>
<td>IMP-1</td>
<td><strong>F</strong> CGTCATAGCTGCTTCTTTG  <strong>R</strong> CTTGTCATCTTGTGTTAGGCG</td>
<td>232</td>
<td></td>
</tr>
</tbody>
</table>

Note: All primers have the following universal tail which is used as a sequencing primer:

*oF*: GTT TTC CCA GTC ACG ACG TTG TA  
*oR*: TTG TGA GCG GAT AAC AAT TTC

Type 1 fimbriae in commensal *E.coli* derived from healthy by pawel pus z….ect

Specimens collection

All specimens were collected during the beginning of September 2018 to the end of April 2019, One hundred and eighty midstream urine specimens were collected from patients attending hospitals (Baghdad Teaching Hospital/ Medical city; Abn Al Baladi hospital; Imam Ali hospital and Al-Numman hospital, Iskan) hospitals.
The Specimens were collected according to\(^{(13)}\). Urine cultured immediately after collection (from hospital laboratory) by streaking 0.01 ml of urine on Blood agar, MacConkey agar, and Eosin Methylene Blue agarin order to isolate Escherichia coli only.

Estimation of IL-6 ; IL-17 and IL-23 in Serum: according to commercially available kit, using the quantitative sandwich enzyme.

**Extraction of DNA from E.coli and Each bacterium was isolated in this study was subjected to molecular screening study using PCR amplification.**

To determine the phylogenetic groups of all isolates in the current study, five primers were used which are IMP ; NDM-1; KPS ; Omp A and Fim h gens by PCR

**Statistical Analysis:** Data analysis was done by descriptive statistics and using SPSS version 20. values of \( p \leq 0.05 \) were regarded as statistically significant relationships.

**Findings**

The study demonstrated that 14.58% of isolated E. coli which positive for each of Omp -A, IMP- A, NDM-A and Fim- H genes were resistant to carbapenem and the study showed that 30.51% of E. coli isolates with KPC were resistant to carbapenem while other E. coli isolates with negative all genes were resistant to carbapenem (Table 1).

<table>
<thead>
<tr>
<th>Genes</th>
<th>Total No. No.</th>
<th>Carbapenem sensitive</th>
<th>Carbapenem resistance</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Omp - A</td>
<td>Positive</td>
<td>48</td>
<td>41</td>
<td>85.42</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IMP - A</td>
<td>Positive</td>
<td>48</td>
<td>41</td>
<td>85.42</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NDM- A</td>
<td>Positive</td>
<td>48</td>
<td>41</td>
<td>85.42</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fim - H</td>
<td>Positive</td>
<td>48</td>
<td>41</td>
<td>85.42</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>KPC</td>
<td>Positive</td>
<td>59</td>
<td>41</td>
<td>69.49</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

In table 2 the Percentage as 72.92% of isolated E. coli were positive for each of Omp – A, IMP - A, NDM- A and Fim – H genes were occurred in females compared with 27.02% occurred in males and 40% of isolated E. coli with positive for KPC gene were in females with UTI.
Table (2): Distributions of virulence genes according to gender of patients

<table>
<thead>
<tr>
<th>Genes</th>
<th>Total No. No.</th>
<th>Female</th>
<th>Male</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Omp - A Positive</td>
<td>48</td>
<td>35</td>
<td>72.92</td>
<td>13</td>
</tr>
<tr>
<td>Omp - A Negative</td>
<td>12</td>
<td>6</td>
<td>50</td>
<td>6</td>
</tr>
<tr>
<td>IMP - A Positive</td>
<td>48</td>
<td>35</td>
<td>72.92</td>
<td>13</td>
</tr>
<tr>
<td>IMP - A Negative</td>
<td>12</td>
<td>6</td>
<td>50</td>
<td>6</td>
</tr>
<tr>
<td>NDM- A Positive</td>
<td>48</td>
<td>35</td>
<td>72.92</td>
<td>13</td>
</tr>
<tr>
<td>NDM- A Negative</td>
<td>12</td>
<td>6</td>
<td>50</td>
<td>6</td>
</tr>
<tr>
<td>Fim - H Positive</td>
<td>48</td>
<td>35</td>
<td>72.92</td>
<td>13</td>
</tr>
<tr>
<td>Fim - H Negative</td>
<td>12</td>
<td>6</td>
<td>50</td>
<td>6</td>
</tr>
<tr>
<td>KPC Positive</td>
<td>59</td>
<td>40</td>
<td>83.33</td>
<td>19</td>
</tr>
<tr>
<td>KPC Negative</td>
<td>1</td>
<td>1</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

In table showed that 10, 50% of isolated E. coli were positive for each of Omp- A, IMP - A, NDM- A and Fim – H genes were occurred in the age group 15-24 year and no isolate were form patients above 54 year.

Table (3): Distributions of virulence genes according to age of patients

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Omp – A, Fim – H, NDM-1 and IMP,</th>
<th>KPC,</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>15-24</td>
<td>6</td>
<td>50</td>
</tr>
<tr>
<td>25-34</td>
<td>3</td>
<td>25</td>
</tr>
<tr>
<td>35-44</td>
<td>2</td>
<td>16.67</td>
</tr>
<tr>
<td>45-54</td>
<td>1</td>
<td>8.33</td>
</tr>
<tr>
<td>&gt;54</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td>P. value</td>
<td>&gt;0.05 (NS)</td>
<td></td>
</tr>
</tbody>
</table>

In table 4, the highest mean levels IL-6 and IL-17 was recorded in UTI patients infected with E. coli positive for each of Omp- A, IMP- A, NDM- A and Fim- H comparing with patients infected with E. coli negative to these genes.
### Table (4): Relation of virulence genes with interleukin IL-6, IL-17 and IL-23 levels

<table>
<thead>
<tr>
<th>GENE Patients</th>
<th>Interleukins levels (IL)</th>
<th>N</th>
<th>(Mean ± S.D)</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Omp - A</td>
<td>IL- 6</td>
<td>48</td>
<td>112.9 ±96.2</td>
<td>N.S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>178.1±128.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL- 17</td>
<td>48</td>
<td>405.6±340.2</td>
<td>N.S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>614.0 ±533.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL- 23</td>
<td>48</td>
<td>361.0±239.9</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>111.1±92.4</td>
<td></td>
</tr>
<tr>
<td>Fim - H</td>
<td>IL- 6</td>
<td>48</td>
<td>112.9 ±96.2</td>
<td>N.S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>178.1±128.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL- 17</td>
<td>48</td>
<td>405.6±340.2</td>
<td>N.S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>614.0 ±533.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL- 23</td>
<td>48</td>
<td>361.0±239.9</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>111.1±92.4</td>
<td></td>
</tr>
<tr>
<td>KPC</td>
<td>IL- 6</td>
<td>59</td>
<td>127.4±120.8</td>
<td>N.S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>41.8 ±none</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL- 17</td>
<td>59</td>
<td>452.3±391.6</td>
<td>N.S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>151.2± none</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL- 23</td>
<td>59</td>
<td>510.4 ±1028.8</td>
<td>N.S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>572.143 ±none</td>
<td></td>
</tr>
<tr>
<td>IMP</td>
<td>IL- 6</td>
<td>48</td>
<td>112.9 ±96.2</td>
<td>N.S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>178.1±128.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL- 17</td>
<td>48</td>
<td>405.6±340.2</td>
<td>N.S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>614.0 ±533.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL- 23</td>
<td>48</td>
<td>361.0±239.9</td>
<td>N.S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>111.1±92.4</td>
<td></td>
</tr>
<tr>
<td>NDM-1</td>
<td>IL- 6</td>
<td>48</td>
<td>112.9 ±96.2</td>
<td>N.S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>178.1±128.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL- 17</td>
<td>48</td>
<td>405.6±340.2</td>
<td>N.S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>614.0 ±533.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL- 23</td>
<td>48</td>
<td>361.0±239.9</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>111.1±92.4</td>
<td></td>
</tr>
</tbody>
</table>
Discussion

Carbapenem-resistant Enterobacteriaceae (CRE) is an urgent public health problem worldwide. These multidrug-resistant organisms exhibit resistance to most, if not all, available antibiotics today and are associated with considerable mortality.(14) In one study, approximately 32% of patients with bloodstream infections caused by carbapenemase-producing CRE died within 14 days.(15) SO (16) Explain the rapid spread of CRE is due to the clonal and plasmid-mediated dissemination of clinical carbapenem-resistant strains,(17), Whilst (18) found that 32.2% of UTI patients with carbapenem-resistant E. coli were females.

Cytokines IL-6 and IL-8 are mediators of inflammation in response to bacterial infection, and when measured in plasma or serum these may be used as early biomarkers of infection.(19) IL-6 and IL-8 levels are also known to be elevated in the urine of patients with UTI, whereas reportedly none are measurable in the urine of healthy controls. The high levels of interleukin 6 in women with acute UTI contribute to increase body temperature and stimulate the production of the C-reactive protein and indicating systemic response of the body toward infection.(18).Moreover, (20) reported higher levels of IL-6 and IL-8 in urine in patients with UTI caused by fimbriated E. coli compared to patients with UTI caused by non-fimbriated E. coli. On the other hand, elevated level of IL-17 in addition to IL-6 in patients with UTI as early innate reaction. Additionally, in murine model that both IL-17 and IL-23 enhance the body to eradicate uropathogenic E. coli.(21). The early innate responses include bacterial expulsion, urothelial exfoliation, and bladder inflammation that is characterized by the production of the pro-inflammatory cytokine interleukin 6 (IL-6), granulocyte chemotactic cytokines such as IL-8, the hormone granulocyte colony stimulating factor (G-CSF), and the T cell-associated, pro-inflammatory cytokine IL-17A (22).

Carbapenem resistance may also be due to AmpC type enzymes or ESBLs along with imperme ability of the membrane (23).Membrane imperme ability can be linked to modifications or absence of OmpC and/or OmpF porin channels or presence of drug efflux pumps (24).

Conclusion

1- levels of interleukin( IL-23 ) were increased significantly (P<0.05) in UTI patients who positive with CRP test compared with CRP negative , also IL-17 elevated moderately in patients with CRP positive while no difference in level of IL-6 between the two groups.

2- Carbapenem-resistant E. coli correlated with founded each of genes ( Omp -A, IMP- A, NDM-A and Fim- H ) , whilst highest correlated between Carbapenem-resistant E. coli with KPC.

Conflict of Interest: Non

Source of Findings: Non

Ethical Clearance: Non

References


11) Veldhoen, M., and B. Stockinger. 2006. a ‘Jack of all trades’: the link with pro-inflammatory IL-17-producing T cells. Trends Immunol. 27:358-361


Evaluation of KV Reduction on image quality in OPG X-Ray

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Abstract

Panoramic radiographs are widely used to obtain an overall survey of the maxillofacial complex. One of the advantages is the reduction in radiation dosage compared with complete intraoral radiography. Digital imaging was first introduced in dentistry for intra-oral radiography, but is now widely available for panoramic radiography. Many studies have demonstrated that it is possible to achieve a degree of dose reduction in digital panoramic radiography.

Keywords: K V, Reduction; image quality; OPG X-Ray

Introduction

In all aspect of radiography digital images have become the new way of view radiographic data to the monitor[1]. In the daily practice of dentistry panoramic radiography takes the second place in vital to intraoral radiography, however, panoramic imaging may be more useful to patients because it offers both excellent anatomical assessment and excellent evaluation of mandible fractures, tooth development and maxillary sinus disorders[2].

The ionizing radiation is an investigative method and the biological effects of radiation have established so much accuracy in the past that the radiation exposure has come to be a public attention fear. According to the ALARA principle (As Low As Reasonably Achievable) which holds that the amount of information must be acquired with the smallest possible amount of radiation[3]. The exposure reach of various digital systems has been described but we still lack information regarding benefit exposure which can produce diagnostically appropriate images[4]. The decrease of radiation dose is considered as one of the most important benefits of digital radiography. It is however, debatable whether digital radiography really offers benefit to intraoral and extra-oral imaging. The conventional extra-oral radiography is depended on screen-film cassette, intensifying screen provides a major dose decrease in comparison to non-screen film-based imaging. Gaining the image digitally, hence, does not enhance much to the dose lessening in extraoral imaging [5]. It is well-known that early detection and intervention of misdirected development can prevent substantial and difficult corrective treatments at later stages and that early detection and intervention most often depend upon radiographic examination. More generally for any group of dental patients, good oral health is not possible without the use of x-ray; so then how to balance between these good diagnostic goals and the hazards of achieving them[6]. The patient dose has also been reported as the effective dose E, this method of reporting resulted from the inability to make direct comparisons between radiographic techniques themselves and background radiation exposure in terms of dose because of the limited area of the body exposed during diagnostic radiology. It is only through the E that possible adverse effects from irradiation to a limited portion of the body can be compared with possible adverse effects from irradiation of the whole body[7]. The special effects of low dose radiation are demonstrable only as a statistical increase in the frequency of normally occurring disease states among the general population[8]. The grade of danger that may be related to exposure to ionizing radiation and may be expressed in two ways; equivalent natural exposure and probability of stochastic effects[9]. From dental radiography, the main hazard is radiation-induced cancer because of low doses exposure[10]. The dentist must use professional judgment when prescribing diagnostic radiograph for dental patients. Diagnostic radiography must be only utilized after clinical examination taking into consideration

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patients' history, dental and general health needs[11]. Every patient must be assessed for dental radiographs on an individual basis[12]. The clinician is advised to use the fastest film type available to minimize patients exposure. Two distances commonly used for intraoral radiography; these are 8 inches and 16 inches[13]. The tissue area which are exposed to the primary beam must not exceed the minimum coverage and should be consistent with clinical feasibility[14]. The collimation is utilized to confine the shape and size of the x-ray beam and to decrease the patient exposure. Patient exposure and dose are reduced when an x-ray beam is properly filtered. Filtration improves the quality of the beam by removing long-wavelength, low energy x-ray so that the mean energy of the beam is increased such that x-rays constituting the beam are nearly all in the use full diagnostic energy range [15]. Leaded thyroid collars are strongly suggested, though scatter radiation to the patient’s abdomen is very low, leaded aprons must be utilized to diminish patient’s exposure to radiation. The lead apron reduces exposure of the reproductive organs and the haematopoietic tissues and organs. The advent of digital imaging has developed radiology due to the improvement of computing systems for image retrieval and transmission and technologic innovation in image acquisition processes[9]. Panoramic radiography is a radiographic technique that creates a single image of the structures in the face, including both maxillary and mandibular arches and their supporting structure[16]. The panoramic radiograph is an excellent means of dental identification when utilized with the Polaroid photograph of the dentition[17]. The Paatero in 1954, introduced the orthopantomograph, the apparatus of panoramic tomography of the maxilla and mandible. It has become widely used in otorhinolaryngology, for radiographic examination of the maxillofacial region[18]. The panoramic radiograph permits professionals to observe a large area of the maxilla and mandible on a single image. The aim of the study was to evaluate the effect of reduction of kilo voltage on interruption of certain anatomical landmark in O.P.G x-ray.

**Patients and Materials**

The Planmeca Proline digital panoramic x-ray machine was used in this study with Dimax3 software, the machine is manufactured by planmeca and has the following specifications [19]: X-ray tubes / focal spot size : D-052SB/0.5* 0.5 mm ; Target angle: 5° ; Total filtration: 2.5 mm Al ; Anode voltage: 60-80 kv ±2.5kv ; Anode current: 4-12 mA ±1.0 mA ; Exposure time: 2.5-18 s as indicated ±10% ; SID : 480 mm ; Magnification: constant 1.2 ; Sensor height: 136 mm ; Line voltage:100,117, 220-230, 240v ; Regulation: automatic ±10% ; Line current: max. 8A at 230v, 15 A at 100v ; Weight: 108 kg ; Cooling period: automatically controlled

**Methods**

After consultation with the engineer responsible about the standardization and maintenance of the x-ray machine, all the subjects were examined radiographically by using digital panoramic radiography machine. Two digital panoramic images were obtained for each subject at 5 minut es interval, the first image was with standard exposure setting, which is recommended by the manufacturer. The second image was taken to the subject with reduced tube current by 50% and so each subject had two digital images. The kVp was kept constant throughout the study; all images were obtained by one radiographer. These tandardization of subject position after first image taking in order to take the second image was achieved by lines drawn on the face to coincide with the horizontal and vertical light beams on the machine. In addition a line was drawn on the floor for the feet position[19].

**Panoramic procedure**

1-The procedure about to be performed was explained for the patient.

2-All objects like eyeglasses, ear rings, necklaces hearing aids, and hair pins, complete or partial dentures should be removed.

3-The patient was advised to stand or sit with the back straight and erect.

4-The patient was instructed to use the plastic bite-block to be bitten on so that the lower and upper anterior teeth were located in an end to end position in the groove (notch), which is on the bite block. This groove is utilized to align the teeth in the focal trough, light was positioned between the canine and lateral incisor of the patient.

5-The middle sagittal plane was positioned vertical to the floor so that the patients head not be tipped or tilted to avoid image distortion.

6-The Frankfort plan was positioned parallel with the floor, so that the occlusal plane is locate dat the correct angle [19].
Finding

Two images for each subject were assessed by the two examiners for evaluation of the fourteen anatomical landmarks. Each examiner gives the score of comparison of each landmark independently and separately.

1. The Sample

This study was based on the analysis of 20 subjects, 10 male and 10 female. The age distribution was between 20-30 years old. For each subject we have two digital panoramic images. There were unacceptable images for viewing for seven of the subjects.

So those seven subjects with their images were excluded from the sample. The seven excluded subjects consisting 14% of the total sample. So the sample that has been examined is 20 samples.

2. Rating scores for male sample by the first examiner

The results show that scores were distributed between equal and worse score with the higher number and percentage for the equal score. For the equal score, 13 landmarks were above 90%. For the worse score, 3 landmarks recorded with zero worse score with higher number and percentage of worse score in one landmark. There was no reading for other scores, Table (1).

<table>
<thead>
<tr>
<th>Anatomical landmarks</th>
<th>Rating score</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>worse</td>
<td>Equal</td>
</tr>
<tr>
<td>Nasal septum</td>
<td>No.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Percentage</td>
<td></td>
</tr>
<tr>
<td>Floor of maxillary antrum</td>
<td>No.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Percentage</td>
<td></td>
</tr>
<tr>
<td>Nasal cavity</td>
<td>No.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Percentage</td>
<td></td>
</tr>
<tr>
<td>Zygomatic arch</td>
<td>No.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Percentage</td>
<td></td>
</tr>
<tr>
<td>Mastoid process</td>
<td>No.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Percentage</td>
<td></td>
</tr>
<tr>
<td>Styloid process</td>
<td>No.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Percentage</td>
<td></td>
</tr>
<tr>
<td>Glenoid fossa</td>
<td>No.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Percentage</td>
<td></td>
</tr>
<tr>
<td>Head of condyle</td>
<td>No.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Percentage</td>
<td></td>
</tr>
<tr>
<td>Mental foramen</td>
<td>No.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Percentage</td>
<td></td>
</tr>
<tr>
<td>Inferior border of mandible</td>
<td>No.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Percentage</td>
<td></td>
</tr>
<tr>
<td>Hyoid bone</td>
<td>No.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Percentage</td>
<td></td>
</tr>
<tr>
<td>Inferior dental canal</td>
<td>No.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Percentage</td>
<td></td>
</tr>
<tr>
<td>molars</td>
<td>No.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Percentage</td>
<td></td>
</tr>
<tr>
<td>Incisors</td>
<td>No.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Percentage</td>
<td></td>
</tr>
</tbody>
</table>
3. Rating scores for female sample by the second examiner

The results show that scores were distributed between the equal and worse score with higher number and percentage for equal score. The equal scores were above 90% in 13 of the landmarks, also there was no reading for worse score is recorded for 12 landmarks and better score is 13. There was no reading for other scores recorded. (Table 2).

<table>
<thead>
<tr>
<th>Table (2): Number and percentage of rating scores of female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anatomical landmarks</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Nasal septum</td>
</tr>
<tr>
<td>Percentage</td>
</tr>
<tr>
<td>Floor of maxillary antrum</td>
</tr>
<tr>
<td>Percentage</td>
</tr>
<tr>
<td>Nasal cavity</td>
</tr>
<tr>
<td>Percentage</td>
</tr>
<tr>
<td>Zygomatic arch</td>
</tr>
<tr>
<td>Percentage</td>
</tr>
<tr>
<td>Mastoid process</td>
</tr>
<tr>
<td>Percentage</td>
</tr>
<tr>
<td>Styloid process</td>
</tr>
<tr>
<td>Percentage</td>
</tr>
<tr>
<td>Glenoid fossa</td>
</tr>
<tr>
<td>Percentage</td>
</tr>
<tr>
<td>Head of condyle</td>
</tr>
<tr>
<td>Percentage</td>
</tr>
<tr>
<td>Mental foramen</td>
</tr>
<tr>
<td>Percentage</td>
</tr>
<tr>
<td>Inferior border of mandible</td>
</tr>
<tr>
<td>Percentage</td>
</tr>
<tr>
<td>Hyoid bone</td>
</tr>
<tr>
<td>Percentage</td>
</tr>
<tr>
<td>Inferior dental canal</td>
</tr>
<tr>
<td>Percentage</td>
</tr>
<tr>
<td>molars</td>
</tr>
<tr>
<td>Percentage</td>
</tr>
<tr>
<td>Incisors</td>
</tr>
<tr>
<td>Percentage</td>
</tr>
</tbody>
</table>
4. Rating scores for total sample by the first examiner

The result represents the total summation of both male and female sample by the first examiner. There was a distribution of the result between equal and worse score with higher number and percentage of equal score. For the equal score, 13 of the landmarks were above 90%, for the worse score, only 4 landmarks have the higher number. There were no readings for other scores (better, much better, much worse). Table (3):

Table (3): Number and percentage of rating scores of total sample

<table>
<thead>
<tr>
<th>Anatomical landmarks</th>
<th>Rating score</th>
<th></th>
<th></th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>worse</td>
<td>Equal</td>
<td>better</td>
<td></td>
</tr>
<tr>
<td>Nasal septum</td>
<td>No.</td>
<td>3</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>Percentage</td>
<td>15.0%</td>
<td>80.0%</td>
<td>5.0%</td>
<td></td>
</tr>
<tr>
<td>Floor of maxillary antrum</td>
<td>No.</td>
<td>11</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Percentage</td>
<td>55.0%</td>
<td>40.0%</td>
<td>5.0%</td>
<td></td>
</tr>
<tr>
<td>Nasal cavity</td>
<td>No.</td>
<td>1</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>Percentage</td>
<td>5.0%</td>
<td>15.0%</td>
<td>80.0%</td>
<td></td>
</tr>
<tr>
<td>Zygomatic arch</td>
<td>No.</td>
<td>3</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>Percentage</td>
<td>15.0%</td>
<td>65.0%</td>
<td>20.0%</td>
<td></td>
</tr>
<tr>
<td>Mastoid process</td>
<td>No.</td>
<td>2</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>Percentage</td>
<td>10.0%</td>
<td>80.0%</td>
<td>10.0%</td>
<td></td>
</tr>
<tr>
<td>Styloid process</td>
<td>No.</td>
<td>12</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Percentage</td>
<td>60.0%</td>
<td>35.0%</td>
<td>5.0%</td>
<td></td>
</tr>
<tr>
<td>Glenoid fossa</td>
<td>No.</td>
<td>1</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>Percentage</td>
<td>5.0%</td>
<td>90.0%</td>
<td>5.0%</td>
<td></td>
</tr>
<tr>
<td>Head of condyle</td>
<td>No.</td>
<td>3</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>Percentage</td>
<td>15.0%</td>
<td>80.0%</td>
<td>5.0%</td>
<td></td>
</tr>
<tr>
<td>Mental foramen</td>
<td>No.</td>
<td>2</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>Percentage</td>
<td>10.0%</td>
<td>75.0%</td>
<td>15.0%</td>
<td></td>
</tr>
<tr>
<td>Inferior border of mandible</td>
<td>No.</td>
<td>0</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Percentage</td>
<td>0.0%</td>
<td>50.0%</td>
<td>50.0%</td>
<td></td>
</tr>
<tr>
<td>Hyoid bone</td>
<td>No.</td>
<td>2</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>Percentage</td>
<td>10.0%</td>
<td>80.0%</td>
<td>10.0%</td>
<td></td>
</tr>
<tr>
<td>Inferior dental canal</td>
<td>No.</td>
<td>2</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>Percentage</td>
<td>10.0%</td>
<td>75.0%</td>
<td>15.0%</td>
<td></td>
</tr>
<tr>
<td>molars</td>
<td>No.</td>
<td>2</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>Percentage</td>
<td>10.0%</td>
<td>75.0%</td>
<td>15.0%</td>
<td></td>
</tr>
<tr>
<td>Incisors</td>
<td>No.</td>
<td>-</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td>Percentage</td>
<td>-</td>
<td>-</td>
<td>100.0%</td>
<td></td>
</tr>
</tbody>
</table>
Figure (1): first image at standard exposure tube current as recommended by Manufacturer

Figure (2): second image at 15% reduction of tube current (mA)
Table 4: number and percentage of each individual score of total sample

<table>
<thead>
<tr>
<th>Rating score</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Better</td>
<td>1</td>
<td>5.0</td>
</tr>
<tr>
<td>Equal</td>
<td>16</td>
<td>80.0</td>
</tr>
<tr>
<td>Worse</td>
<td>3</td>
<td>15.0</td>
</tr>
<tr>
<td><strong>total</strong></td>
<td>20</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Discussion

Past studies have demonstrated that it is possible to achieve a degree of dose reduction in digital panoramic radiography without impairment of image quality. The aims of the present clinical study were to evaluate the effect of 15% dose reduction with the digital panoramic radiography unit on subjective image quality and interpretation performance hoping to achieve the desirable amount of information with the smallest amount of radiation.

Conclusions

This study has shown that in digital panoramic radiography, a dose reduction of 15% can be achieved while maintaining satisfactory image quality and interpretation performance.

Recommendations:

1- Design to assess the perfection of interpretation of certain anatomical landmarks at reduced dose digital panoramic radiography.

2- The capability of minimizing hazardous effects of radiation through studying the effect of dose reduction on human living tissue in digital panoramic radiography.

Conflict of Interest: non

Source of Findings: self

Ethical Clearance: This research was carried out with the patients.

References


4- Borg E, A Attaelmanan and HG Grondahl. Subjective image Quality of solid state and photostimulable phosphor systems for digital intraoral radiography. 2000.29, 70-75.


7- Bushong SC. Radiologic science for technologists: Physics, Biology and protection. 2001. 7th ed; P 42 – 44.


Population Prevalence of Asthma in Aljiza District

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1Department of Pharmacy, Faculty of Pharmacy, Middle East University, Amman, Jordan

Abstract

Background: Asthma is a condition in which your airways narrow and swell and produce extra mucus. Objective: In this study we determine the prevalence of asthma among the population of Aljiza District, Jordan. In order to determine the prevalence of asthma in Aljiza District (Amman, Jordan), Method: a questionnaire was distributed to 4200 respondents of different age and gender. Results and Conclusions: The responses were reviewed and the incidences were determined. From the 4200 questionnaires by respondents 131 person (3.1%) suffered from physician-diagnosed asthma. No attempt was made to confirm the diagnosis and the results relied upon responses to questionnaire. The prevalence of asthma in adult females was higher than that of males in all groups. Adult females and males showed a significant priority on other age groups, and children, 5-10 years group have higher prevalence than children, 10-15 years group, in both males and females. In order of frequency, the triggering factors were common cold, dust, allergens, smoke, exercise, and chest infection. A relationship between asthma and paternal smoking, family history and to a lesser extent recurrent chest infections was suggested. The prevalence of asthma in Aljiza District is less than that reported in the southern and northern parts of Jordan and some Middle East countries. Although our results show a low prevalence of asthma, it still is a health problem among children and the adults in Aljiza District.

Keywords: Asthma, population prevalence, Aljiza District, Jordan

Introduction

Asthma is one of the most common chronic respiratory diseases and is a major health problem worldwide, affecting people of all ages, genders and religions (1). The prevalence of asthma may be as high as 334 million according to a report from the Global Asthma Network published in 2014 (2).

The disease represents a significant burden at life not only in term of morbidity and quality of life, but also in terms of health cost, especially in developing countries (3, 4).

Though most of the available information regarding the prevalence of asthma indicates that West European countries have some of the highest prevalence rates of asthma in the world, nevertheless Latin American, Asian, Middle Eastern and some Arab countries also showed a relatively high prevalence of this disease (4, 5). Moreover, results on the prevalence of asthma in Arab countries suggest that the highest rate was reported among people who lived in desert (6) and lowest is among people living in urban areas (7).

In Jordan and though the disease is increasing recently there is a limited information on the prevalence of asthma in many parts of the country (8). The aim of the present study is to investigate the prevalence and severity of asthma and asthma related symptoms in Aljiza District.

Material and Method

Aljiza District is located about 50 km. south of Amman with a population of about 60000 inhabitants and also the study protocol conformed to the ethical guidelines of the 1975 Helsinki Declaration and the approval was obtained from ethical committee of Middle East University-Amman-Jordan.
The design of this study was cross-sectional. It was carried out among people living in this area to include a random sample of school children, university students and families. For the school children a self-administered questionnaire was distributed to parents via their children, who returned the questionnaire after it had been completed by one of their parents. The questionnaires asked for the following details: age and sex, chest diseases, allergic rhinitis, family history of asthma, parental smoking.

The data were analyzed using statistical package for social science (SPSS) version 23. Descriptive statistics. Chi – square test (X2) was used in this study on a level of significance less than (P <0.05).

Results

A survey was conducted on a sample of 4200 individuals. For this purpose a questionnaire was prepared and distributed. The completed questionnaires include 2500 males and 1700 females. The questionnaires revealed that 131 subjects (3.1%) have physician diagnosed asthma including 71 males (2.8 %) and 60 females (3.5%). The results are presented in Table 1.

Table II shows that in all age groups the prevalence in females is higher than that in males, and that it is significantly higher in adult female than in the other two female age groups (5-10 yrs.) and (10-15 yrs.). The same trend is observed within male prevalence asthmatic groups where prevalence is significantly higher in the adult group than in the 5-10 yrs. and 10-15 yrs. groups. The results also show that children (5-10 yrs.) have higher prevalence than that of (10-15 yrs.) and this trend is consistent in both female and male groups.

Most of asthma episodes appeared to be triggered by common cold (64.1%), dust (31.3%) and allergens (30.5%) and to less extent smoke (14.5%), exercise (12.2%) and chest infections (3.8%) (Table III).

Table IV shows the relationship between asthma and the risk factors studied. The results revealed that paternal smoking (42.7%), family history (24.4%) and recurrent chest infections (19%) are the most significant factors associated with this disease. (Table IV).

<table>
<thead>
<tr>
<th>Table 1: prevalence of asthma in Aljiza District, Jordan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female No. (%)</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>1700</td>
</tr>
<tr>
<td>Physician diagnosed</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table II: distribution of asthma patients among age groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age groups</td>
</tr>
<tr>
<td>5-10 yrs.</td>
</tr>
<tr>
<td>No. (%)</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Male</td>
</tr>
</tbody>
</table>
Table III: Triggering factors of asthma among 131 individuals

<table>
<thead>
<tr>
<th></th>
<th>Female No. (%)</th>
<th>Male No. (%)</th>
<th>Total No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common cold</td>
<td>41 (68.3)</td>
<td>43 (60.6)</td>
<td>84 (64.1)</td>
</tr>
<tr>
<td>Smoking</td>
<td>7 (11.7)</td>
<td>12 (16.9)</td>
<td>19 (14.5)</td>
</tr>
<tr>
<td>Allergens</td>
<td>22 (36.7)</td>
<td>18 (25.4)</td>
<td>40 (30.5)</td>
</tr>
<tr>
<td>Exercise</td>
<td>8 (13.3)</td>
<td>8 (11.3)</td>
<td>16 (12.2)</td>
</tr>
<tr>
<td>Chest infection</td>
<td>2 (3.3)</td>
<td>3 (4.2)</td>
<td>5 (3.8)</td>
</tr>
<tr>
<td>Dust</td>
<td>19 (31.7)</td>
<td>22 (31)</td>
<td>41 (31.1)</td>
</tr>
</tbody>
</table>

Table IV: The risk factor for physician diagnosed asthma

<table>
<thead>
<tr>
<th>Personal history</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recurrent chest infection</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>25 (19)</td>
</tr>
<tr>
<td>Absent</td>
<td>106 (81)</td>
</tr>
<tr>
<td>Allergic rhinitis</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>5 (3.8)</td>
</tr>
<tr>
<td>Absent</td>
<td>126 (96.2)</td>
</tr>
<tr>
<td>Family history</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>32 (24.4)</td>
</tr>
<tr>
<td>Absent</td>
<td>99 (75.6)</td>
</tr>
<tr>
<td>Maternal smoking</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>10 (8.3)</td>
</tr>
<tr>
<td>Absent</td>
<td>121 (91.7)</td>
</tr>
<tr>
<td>Paternal smoking</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>56 (42.7)</td>
</tr>
<tr>
<td>Absent</td>
<td>75 (57.3)</td>
</tr>
</tbody>
</table>

Discussion

The primary objective of this study was to estimate the prevalence of asthma in a semi-desert area (Aljiza). The result of this study revealed an overall prevalence of asthma of 3.1% which is lower than that reported from Jordan and many other Middle East countries (1, 9-13). However, a study carried out in Anatolia, Turkey showed that a prevalence of asthma as low as 1% in age group of (15-29 yrs.) and 2.6% in the (13-49) age groups. This is a low a low prevalence and is aligned with our findings (14).

Result on asthma from different countries and localities have consistently shown a high prevalence of this disease in women during adulthood (1, 10, 15, 16). Consistent with this, our result revealed a similar finding in that a significant higher prevalence in women compared to men (p>0.05). Though there are many explanations for this phenomenon no single explanation can fully explain the gender difference. Among these hypotheses female hormones might play a role in inducing this higher prevalence (1).
Concerning age groups, the present study showed that children of age group 5-10 yrs. was slightly higher than age group of 10 to 15 yrs. group. This is in agreement with results reported by other authors (9,17). Furthermore, our findings concerning gender difference showed that the rate of physician diagnosed asthma was higher in female age group 5-10 yrs. and 10-15 yrs. respectively. This is not in agreement with previous studies in Jordan and elsewhere (9, 12, 18).

Several triggering factors were examined in this study. Two among them, namely common cold and allergens show highly significant association with the disease; others like smoking and dust also have a role in triggering asthmatic attack. This confirmed earlier findings from different localities in Jordan and elsewhere (9, 14).

Finally, with regard to risk factors paternal smoking and family history appears to be the most important risk factors in this survey, (42.7 %) and (24%) respectively. This is an agreement with Weitzman et al. (1990) findings (18).

Though the present results indicated a low prevalence of asthma to confirm these results a further study is needed in the area.

**Conclusion**

The observed prevalence of asthma in Aljiza is 3.1 % which is comparatively lower than the reported prevalence in many parts of Jordan and Middle East countries. Though the present results indicated a low prevalence of the disease still there is a belief that asthma and wheezing have a negative impact on health and the quality of life and therefore further studies are needed to obtain a reliable data in the prevalence of asthma in both children and adults by using a Standard European community Respiratory Health Survey (ECRHS) questionnaire and tools.

**Acknowledgement:** The authors are grateful to the Middle East University, Amman, Jordan for the financial support granted to cover the publication fee of this research article.

**Conflict of Interest:** None

**Ethical Clearance:** The study protocol conformed to the ethical guidelines of the 1975 Helsinki Declaration and the approval was obtained from ethical committee of Middle East University-Amman-Jordan.

**References**


Rituximab in the Treatment of Refractory Myasthenia Gravis: Studying the Outcomes Using MMT Score And Need for Plasmapheresis in Baghdad Teaching Hospital

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\textsuperscript{1}Lecturer, Department of Clinical Pharmacy, College of Pharmacy, Mustansiriyah University, Iraq, \textsuperscript{2}Consultant Neurologist, Baghdad Teaching Hospital, Iraq, \textsuperscript{3}Clinical Pharmacy Specialist, Intensive Care Unit, Baghdad Teaching Hospital, Iraq, \textsuperscript{4}Bactriologist, Baghdad Health Directorate - Al-Karkh, Iraq

Abstract

Introduction: Myasthenia gravis (MG) is an uncommon autoimmune disease that is caused by acetylcholine receptor antibodies (AChRA) at the neuromuscular junction. Its pathogenesis involves complement fixing antibodies directed against acetylcholine receptors, muscle-specific tyrosine kinase or low density lipoprotein receptor–related protein.

Method: A descriptive case series study investigating rituximab in refractory MG was conducted in Baghdad teaching hospital. Patients who were included in the study had received a regimen of rituximab that was deemed appropriate by their treating physician.

Results: A total of 24 patients with mean disease duration of 6.3 years ±3.6 standard deviation were included in this study. The results showed that the average age of patients was 33.3 years ±10.1 SD and 62.5% was females. All twenty four patients showed an obvious improvement in clinical status after finishing the follow-up period. The results demonstrated from all patients that enrolled in our study, only one patient that was on 14 sessions of plasmapheresis was in need for plasmapheresis after induction of rituximab and this difference was statistically significant.

Conclusion: There were a great clinical improvement as shown with MMT score in addition to discontinuation of plasma exchange treatments following treatment with rituximab.

Keywords: Rituximab, Myasthenia gravis, Refractory, plasmapheresis

Introduction

Myasthenia gravis (MG) is an uncommon autoimmune disease that is caused by acetylcholine receptor antibodies (AChRA) at the neuromuscular junction\textsuperscript{[1]}. Its pathogenesis involves complement fixing antibodies directed against acetylcholine receptors, muscle-specific tyrosine kinase or low density lipoprotein receptor–related protein\textsuperscript{[2]}. Muscle weakness, with abnormal fatiguability, and improvement after rest, characterize myasthenia gravis. Symptoms tend to be worse at the end of the day, and after repetitive use of muscles for a particular task\textsuperscript{[3]}. Commonly, MG affects the ocular muscles first and this results in ptosis and diplopia\textsuperscript{[4]}. Many studies was done to clarify the meaning of refractory myasthenia gravis and use specific standards like failed response to standard duration and doses of traditional treatment with immunosuppressive drugs, undesirable adverse reactions to conventional therapy, the need for frequent use of short period therapies such as IV Immunoglobulin and plasmapheresis, and/or suffering patients from repeated myasthenic crises\textsuperscript{[5]}. It can occur at any age but most commonly affects women under the age of 40 and men over the age of 60\textsuperscript{[6]}. There are only a few studies on the epidemiology of myasthenia gravis based on complete populations\textsuperscript{[7]} prevalence rates have increased to about 20 per 100,000 in the US population\textsuperscript{[8]}. There are a deficit in epidemiological data on MG in Arab countries. The incidence rate reported from Libya is similar to the worldwide incidence rate of 5.3 per million person-years estimated in a systematic review of population-based studies, and showed a higher
The neuromuscular abnormalities in MG are thought to be an autoimmune response related to specific anti-AChR antibodies, but how the autoimmune response is started and preserved in MG is not fully understood \(^{[10]}\). AChR antibodies mostly belong to the IgG1 and IgG3 subclasses, which stimulate the complement cascade to destruct the postsynaptic membrane, which may lead to up regulation of inflammatory cytokines \(^{[11]}\). The ultimate goal of treatment is to achieve complete stable remission, defined as no myasthenic symptoms or signs without any ongoing treatment for at least 1 year \(^{[12]}\). Therapies in MG should therefore eliminate patients’ symptoms within the boundaries of adverse events associated with treatment itself \(^{[13]}\).

### Patients and Method

A descriptive case series study examining rituximab in refractory MG was performed in Baghdad teaching hospital. Patients who were included in the study had received a regimen of rituximab that was deemed appropriate by their treating physician. Total of twenty-four patients identified with refractory generalized MG were enrolled in the study. Physical tests were estimated before and after treatment with rituximab. MMT score is used to show clinical improvement. MMT is a procedure for the evaluation of strength of individual muscle or muscles group based upon the effective performance of movement relation to the forces of gravity or manual resistance.

There were no specific criteria used to stage clinical response against no clinical response. The main outcome of the study was the improvement in MMT score and symptoms of patients, with the other outcomes being the change in the frequency of plasma exchange which were done at the estimation of the clinician. Patients that are included in the study are 13 to 90 years old that must have refractory MG, that not respond to glucorticoid and other immunosuppressive therapy. Subjects must be on a stable standard immunosuppressive regimen with no history of thymoma, tumor, infection, or interstitial lung disease on chest CT, MRI, or chest x-ray. Patients that are excluded from the study are patients with history of chronic degenerative, psychiatric, or neurologic disorder other than MG that can produce weakness or fatigue. Female subjects who are premenopausal who are pregnant, breast feeding or not use effective method for contraception. Protocol for Rituximab was administered in a standard dose of 1g. Every cycle is estimated as one infusion for two weeks. The space between cycles was adjusted as 6 months. To see the safety and adverse events, we collected notes from the infusion center and also, a complete blood count and liver function test profiles present in patient’s medical records. SPSS version 22 was used for data entry and analysis. Mean and standard deviation was used to represent the numerical data while the frequency and percentage for categorical data. Independent student T test and the test chi-square (Fischer exact test if not applicable) were used to confirm significance \(p < 0.05\) was considered significant.

### Results

A total of 24 patients with mean disease duration of 6.3 years ±3.6 SD were included in this study. The results showed that the mean age of patients was 33.3 years ±10.1 SD and 62.5% was females. All twenty-four patients showed a marked improvement in clinical status by the end of the follow-up period. Five of the patients underwent a single cycle of rituximab, two received two cycles of rituximab while the remaining received either three or more cycles of rituximab. The results indicated there was no significant difference \((p=0.5)\) in plasmapheresis session that as needed after induction of rituximab when compared according to number of cycle of rituximab in term of < 4 or ≥ 4 cycles, where the results showed that only one patient was in need for plasmapheresis session of those who received less than 4 cycles of rituximab while no one in need for plasmapheresis session of those who received more than 4 cycles of rituximab as seen in table 1.

<table>
<thead>
<tr>
<th>cycle of treatment with rituximab</th>
<th>plasmapheresis session</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>&lt;4</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>≥4</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>
The results demonstrated from all patients that enrolled in our study, only one patient that who was on 14 sessions of plasmapheresis was in need for plasmapheresis after induction of rituximab and this difference was statistically significant (p=0.03) as seen in table 2.

**Table 2: Associations between plasmapheresis sessions pre and post treatment with rituximab**

<table>
<thead>
<tr>
<th>Plasmapheresis session-pre rituximab/no. of patients</th>
<th>plasmapheresis session/post rituximab</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>No sessions (1 patients)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1 session (1 patients)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2 session (2 patients)</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>3 session (5 patients)</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>4 session (6 patients)</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>5 session (3 patients)</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>6 session (1 patients)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>7 session (1 patients)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>8 session (1 patients)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>12 session (1 patients)</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Regarding MMT score the results indicated that the highest frequency (50%) of patients was in score 2 of MMT score before using the rituximab followed by score 3 (33.3%) and lastly the score 1 (16.7%) as seen in table 3.

**Table 3. Frequency of patients according to MMT scores before using rituximab.**

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score-2</td>
<td>12</td>
</tr>
<tr>
<td>Score-3</td>
<td>8</td>
</tr>
<tr>
<td>Score-1</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
</tr>
</tbody>
</table>

After using the rituximab; the highest frequency of patients 12 (50%) was improved to score 5 of MMT followed by score 4 (33.3%) and only 16.7% was in score 3 as seen in table 4.

**Table 4. Frequency of patients according to MMT scores post using rituximab.**

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score-5</td>
<td>12</td>
</tr>
<tr>
<td>Score-4</td>
<td>8</td>
</tr>
<tr>
<td>Score-3</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
</tr>
</tbody>
</table>
The finding revealed that from 4 patients who was with score one before treatment with rituximab, 3 patients was improved to score 4 and 1 to score 5. From 12 patients who were in score 2 before using of rituximab, 4 patients were improved to score 3, 3 patients improved to score 4 and 5 patients to score 5. From 8 patients who were in score 3 before treatment with rituximab, 2 patients were improved to score 4 and 6 patients to score 5. So the results revealed that the frequency of patient who had pretreatment high score of MMT, who were reach the highest score after treatment with rituximab was higher than those who were already had low score before using the rituximab but this difference was non-significant as seen in table 5.

Table 5: Association of MMT pre and post using of rituximab

<table>
<thead>
<tr>
<th>MMT/ before</th>
<th>MMT/after</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score-1(n=4)</td>
<td>Score-3</td>
<td>0</td>
<td>0.0%</td>
<td>3</td>
<td>75.0%</td>
<td>1</td>
<td>25.0%</td>
</tr>
<tr>
<td>Score-2(n=12)</td>
<td>Score-4</td>
<td>4</td>
<td>33.3%</td>
<td>3</td>
<td>25.0%</td>
<td>5</td>
<td>41.7%</td>
</tr>
<tr>
<td>Score-3(n=8)</td>
<td>Score-5</td>
<td>0</td>
<td>0.0%</td>
<td>2</td>
<td>25.0%</td>
<td>6</td>
<td>75.0%</td>
</tr>
</tbody>
</table>

Discussion

In this study of 24 patients that present with refractory generalized myasthenia gravis, we see that rituximab result in a sustained clinical improvement as demonstrated by improved MMT score in addition to reduce frequency or lack of need for plasma exchange treatments as shown with Anderson et al study[14]. At time of this study, all patients were followed for 1 year following infusion with rituximab. The results of this analysis favor the theory that rituximab can be effective for treatment of refractory MG which is compatible with small reports that were done previously as in Zebardast et al and other studies[15,16]. Most patients may need two or more cycles of rituximab for obvious decrease and discontinuation of other immunosupressant, in addition to the attainment of disease remission. As there is no fixed protocol for rituximab use in myasthenia gravis, retreatment is decided based on disease activity and clinical improvement, with a minimum interval between infusions of 6 months which is similar to Peres et al[17]. A perfect protocol has not yet been confirmed, however, some studies used peripheral B-cell count as a guide for retreatment with rituximab and to decrease toxicity and side effects[18,19]. An additional studies are required to recognize the best objective clinical indicators and to fix pharmacokinetics in this kind of patients. Immunoglobulin levels, B-cell counts, titers for antibody would seem the most helpful objectives at this time but in our study we depend on symptoms improvement and MMT score. All patients in this study can tolerate rituximab with no severe hematologic derangements[20,21]. The patients in this study were monitored clinically for rituximab adverse effects while in the infusion center as well as with CBC and LFT at baseline and after each infusion. All patients in this study can tolerate rituximab with no severe hematologic derangements. Infusion reaction is the most common side effect reported in general[22]. There is a need for a larger prospective controlled trial to gain more definitive conclusions about the efficacy of rituximab in the treatment of refractory MG. The strong effect of rituximab in patients with refractory MG in our center as well as in similar studies is promising and suggests that further investigation of this agent in MG is warranted[23,24].

Conclusion

There were a great clinical improvement based on MMT score, in addition to decrease or cutting the use of plasma exchange following treatment with rituximab. Rituximab is an attractive treatment because of its mechanism of action that result in targeting CD20-positive B cells which are engaged in the production of antibodies. Most patients in this study can tolerate rituximab with no sever hematologic derangements and mild infusion reactions were the most common side effects associated with our study.

Conflict of Interest: Nil

Source of Funding: self
Ethical Clearance: verbal consent was obtained from the patient before his enrollment in the study.

Reference:

Evaluating the Antifungal Efficacy of Incorporating Kappa-Carrageenan Powder Into “Heat-Cured, Acrylic-Based Soft Denture Lining Material

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¹Department of Prosthodontics, College of Dentistry, University of Baghdad/Iraq

Abstract
Denture stomatitis is one of the serious problems that are related to the continuous use of soft lining material due to the accumulation of microorganisms. It is caused mainly by fungal growth, especially of Candida albicans. It, therefore, becomes necessary to examine the effectiveness of incorporating an antifungal drug into the soft lining material.

This study was conducted to investigate the efficacy of adding the kappa-carrageenan powder to heat-cured, acrylic-based soft lining material against Candida albicans adherence.

A pilot study was performed to decide the best concentration of kappa-carrageenan to be used with the heat-cured, acrylic-based soft liner. Five percentages by weight of kappa-carrageenan powder (0.5, 1, 1.5, 2, and 2.5 wt.%) were evaluated and compared with the control group (0 wt.%). The Candida albicans adherence test was performed to assess the efficacy of kappa-carrageenan powder in preventing the adherence of Candida albicans. Results showed the 1.5 wt.% and 2 wt.% groups as having the best effect.

The main study included preparing thirty samples and dividing them into three groups based on the percentage of powder of kappa-carrageenan added to the heat-cured acrylic-based soft lining material (control: 0 wt.%; experimental: 1.5 wt.% and 2 wt.%). The purpose of performing Candida albicans adherence test was to evaluate the antifungal efficacy of kappa-carrageenan powder addition, and all the resulted data were analysed using “one-way analysis of variance (ANOVA) and Dunnet T3 post-hoc test at a significance level of $p<0.05$”.

The results of Candida albicans adherence test revealed a highly significant decrease in the values of the adhered Candida albicans cells after incorporation of 1.5 wt.% and 2 wt.% of kappa-carrageenan powder when compared to the control group ($p<0.01$).

The result of this study suggests that the addition of kappa-carrageenan powder is an effective drug against Candida albicans adherence on the surface of the heat-cured acrylic-based soft lining material, and the addition of 2 wt.% is more effective than 1.5 wt.%

Keywords: soft liner, kappa-carrageenan, Candida albicans, denture stomatitis.

Introduction
Denture resilient lining materials that are applied over the intaglio surface of the denture to work as a cushion to absorb the stress generated by occlusal forces and to decrease continuous trauma on the denture-bearing area, making wearing the denture more tolerable to the patients¹.

The ideal soft lining material exhibits a number of properties to ensure maximum benefits for denture wearers; which may include dimensional stability, colour stability, good resiliency, biocompatibility, sufficient bond strength with the denture base material, low water solubility, and resistance to microbial growth².
The colonization of microbes in the soft lining material is one of the serious problems that affect its long-term efficacy. The most common clinical condition associated with this problem is “denture-induced stomatitis”; this condition is mainly caused by *Candida albicans*, which is the most popular fungi responsible for oral infections(3).

Topical antifungal medicaments can be considered as the most common line of treatment for “denture-induced stomatitis”. However, certain difficulties are associated with this type of management, such as the inability of the elderly to deliver the optimum dosage of the drug due to a lack of motor dexterity. To overcome this issue, a suggestion has been made to develop a drug delivery system by incorporating the drug into the denture materials. Furthermore, the development of fungal resistance against the drug due to its continual use for a prolonged time makes it necessary to search for a new effective medicament to be used as an alternative to synthetic drugs(3,4).

Herbal medicines have proved to be an excellent alternative treatment line for the management of oral infections, and this makes it necessary to investigate these products to ensure their biological safety and antifungal properties(5).

Carrageenan is a water-soluble sulphated galactan and major cell-wall component in red algae. It has been identified as possessing high anti-coagulant, anti-oxidant, anti-tumour, and anti-microbial activity(6). In addition, carrageenan has been used for many years as a food additive and in pharmaceutical applications due to its stabilizing, thickening, and emulsifying properties, and it is accepted by the U.S. Food and Drug Administration(FDA) and the World Health Organization(WHO). The recommended usage of carrageenan as an additive is advised to be in the range of 0.005–3%(7). Its suggested that these sulphated polysaccharide are negatively charged molecule which have inhibitory effect through its ability to produce alteration in the cell wall by interacting with the positive charge on the cell surface(8). Thus, this study was conducted to evaluate the effect of kappa-carrageenan against *Candida albicans* adherence to the heat-cured, acrylic-based soft lining material.

### Materials and Method

#### Pilot study

Heat-cured, acrylic-based soft liner(Vertex,Netherlands) was used. Kappa-carrageenan powder(Sigma-Aldrich,Denmark) was added to the powder of the soft liner in six percentages by weight of the powder (0, 0.5, 1, 1.5, 2, and 2.5wt.%). These groups were used to decide the two groups that produced the best effect against *Candida albicans* adherence. Mixing was done according to manufacturer instructions for the control group without the additive (0wt.%), while for the experimental group, the weight of kappa-carrageenan powder was subtracted from the weight of the soft liner powder to maintain an optimum powder/liquid ratio(9). The *Candida albicans* adherence test was conducted; four samples were used for each group. The results of the pilot study demonstrated that 1.5wt.% and 2wt.% produced the best effect against *Candida albicans* adherence to the denture soft lining material, so it was selected for the main study.

The pilot study also assessed the efficacy of using kappa-carrageenan powder in disinfectant form by adding it to the distilled water to be used as an immersion solution for denture disinfection. However, as the powder was added to the distilled water in the room temperature, it formed a gelatin that was thick in consistency; thus, the procedure was excluded from the main study due to a lack of feasibility.

#### The main study

A total of thirty samples were prepared and divided into three groups, with ten samples for each group: control group(C): heat-cured, acrylic-based soft liner without the additive; experimental group(E1): heat-cured, acrylic-based soft liner with 1.5wt.% kappa-carrageenan powder additive; and experimental group(E2): heat-cured, acrylic-based soft liner with 2wt.% kappa-carrageenan powder additive.

#### *Candida albicans* adherence test

**Sample preparation**

A plastic disc measuring 10×2 mm in diameter and thickness, respectively, was fabricated to make the final shape of the soft liner samples used for the *Candida albicans* adherence test(10). These plastic molds were invested in addition-type silicone material (Zermack,Italy); molds with the silicone replica were
then invested in the lower portion of the dental flask with freshly mixed dental stone (Zermack, Italy). Samples of the control group were prepared according to manufacturer instructions for the heat-cured, acrylic-based soft liner (powder/liquid ratio: 1.2 g powder/1 ml monomer), and mixed together using a clean glass container. For experimental samples, the weight of the kappa-carrageenan powder was subtracted from the weight of the soft liner powder to obtain an accurate powder/liquid ratio. An amalgamator device (Perfection Plus, United Kingdom) is used to mix the two powders for 40 seconds to obtain a homogeneous mixture. All samples were cured according to manufacturer instructions using a thermostatically controlled water bath (Lab.tech, Korea) heated to 70°C for 90 minutes; temperature was then raised to 100°C for 30 minutes. After processing, samples were finished with sharp scissors and polished with a fine-grit, silicone polishing bur under continuous water cooling. All samples were stored in distilled water for 24 hours at 37°C before being tested to eliminate any residual monomer.

Isolation and identification of Candida albicans

A patient with signs and symptoms of “denture-induced stomatitis” attending the College of Dentistry/University of Baghdad was selected. The Candida was isolated from the patient’s mouth using a sterile cotton swab and gentle rubbing of the intra-oral lesion, which was cultured on the surface of a “Sabouraud dextrose agar” (SDA; Oxoid, England) plates and incubated at (37°C) for 48 hours.

Candida albicans identification was made using macroscopic examination. The Candida albicans had a pearl-shaped appearance, with a creamy and pasty texture on SDA. A microscopic examination was conducted using a light microscope (Olympus, Japan) and gram stain procedure (Fig. 1) biochemical identification was made using the API-20C-AUX system and API-Candida system.

Candida albicans adherence test

The Candida albicans adherence test was accomplished by preparing a Candida albicans suspension using normal saline, which is equivalent to 0.5 McFarland standards. Then, 0.1% of Candida albicans suspension was added to a test tube containing 0.9% sabouraud dextrose broth (Oxoid, England) using micropipette; samples were immersed in this tube and incubated at (37°C) for 1 hour. After incubation, samples were taken out of the suspension and washed with phosphate-buffered saline for 1 minute, and dried using absorbent paper. Fixation of the adherent cells is done using methanol 80% for 30 seconds, followed by staining with crystal violet stain for 1 minute.

An inverted light microscope (Karl Kolb, Germany) was used to examine the samples using 40× magnification power, with the microscope being connected to a computer. Four standardised fields were examined in each sample.

Statistical analysis

Results of the presented research were analysed using SPSS “version 24 computer software”. The descriptive statistics that have been “made, includes means, standard deviation, and graphical presentation by bar chart”. “Inferential statistics” were also made, including one-way analysis of variance (ANOVA), for comparison means among all groups, and Dunnet T3 multiple comparison tests, which shows the significance between each of the two different groups, with P<0.05 considered significant.

Results:

Evaluating Candida albicans adherence

Results of the Candida albicans adherence test were evaluated by counting the number of adhered Candida on the surface of the control and experimental samples through the use of an inverted light microscope, in which it appeared as round or oval violet cells (Fig. 2). The results for both experimental groups showed lower mean values compared with the control group, with the experimental group (E2) revealing the lowest mean value of Candida albicans (4,910 cells) compared with the control group (C), which had the highest values of adherent cells (36,600 cells) (Fig. 3; Table 1).

Figure 1: Microscopic examination Candida albicans on sabouraud dextrose agar (SDA).
Figure 2: Microscopical image of *candida albicans* on soft liner samples. C) Control samples (without additive), E1) experimental samples (with 1.5 wt.% of kappa-carrageenan additive), and E2) experimental samples (with 2 wt.% of kappa-carrageenan powder additive).

Figure (3): Bar chart showing mean values and standard deviation of *Candida albicans* adherence for control (C) and experimental groups (E1 and E2).

Table (1) Descriptive statistics of *Candida albicans* adherence test.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean</th>
<th>SD</th>
<th>SE</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>36.600</td>
<td>5.119</td>
<td>1.619</td>
<td>30.000</td>
<td>44.300</td>
</tr>
<tr>
<td>E1</td>
<td>9.530</td>
<td>1.027</td>
<td>0.325</td>
<td>8.000</td>
<td>11.300</td>
</tr>
<tr>
<td>E2</td>
<td>4.910</td>
<td>1.101</td>
<td>0.348</td>
<td>3.300</td>
<td>7.000</td>
</tr>
</tbody>
</table>
Results of the one-way ANOVA test were highly significant among all groups ($p<0.01$). Dunnet T3 test showed a highly significant decrease for both experimental groups compared with the control group, and there was also a highly significant difference between the two experimental groups ($p<0.01$); Tables 2 and 3.

**Table 2: Statistical test of *Candida albicans* adherence among groups using one-way ANOVA**

<table>
<thead>
<tr>
<th></th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
<th>Effect size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>5861.285</td>
<td>2</td>
<td>2930.642</td>
<td>308.814</td>
<td>.000 [HS]</td>
<td></td>
</tr>
<tr>
<td>Within Groups</td>
<td>256.230</td>
<td>27</td>
<td>9.490</td>
<td></td>
<td></td>
<td>0.958</td>
</tr>
<tr>
<td>Total</td>
<td>6117.515</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table (3): Multiple comparisons of *Candida albicans* adherence between groups using the Dunnett T3 post hoc test.**

<table>
<thead>
<tr>
<th>(I) Groups</th>
<th>(J) Groups</th>
<th>Mean Difference (I-J)</th>
<th>Sig.</th>
<th>Effect size</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>E1</td>
<td>27.070</td>
<td>.000</td>
<td>HS</td>
</tr>
<tr>
<td></td>
<td>E2</td>
<td>31.690</td>
<td>.000</td>
<td></td>
</tr>
<tr>
<td>E1</td>
<td>E2</td>
<td>4.620</td>
<td>.000</td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

Herbal medicine is considered to be very reliable alternative to antimicrobial drugs, with little or no side effects; for this reason, there is worldwide interest in medicinal plant extracts. Therefore, much research has been conducted regarding their biological safety (4).

Kappa-carrageenan is a sulphated polysaccharide and major cell-wall component in red algae. Carrageenan has been studied recently for its several medicinal benefits, and it has been suggested that it can alter the cell wall by interacting with the positive charges on the cell surface (8).

The *Candida albicans* adherence test was performed because the adherence of *Candida albicans* on the fitting surface of dentures has been proven to be of critical importance in the development and maintenance of denture stomatitis in continuous denture wearing (18).

In this study, the incorporation of kappa-carrageenan into the heat-cured, acrylic-based soft lining material resulted in a decrease in the number of *Candida* cells adhered to the surface of the experimental samples when compared to the control groups.

The effect of kappa-carrageenan on *Candida albicans* is explained by Souza et al. (2018) who stated that these macromolecules possess antifungal activity and induce resistance to fungal organisms. This is in agreement with Soares et al. (2016) who suggested that carrageenan extracts promote morphological alterations in the cell wall of *Candida* species. After the exposure of *Candida* cells to kappa-carrageenan, the chitin cell wall content is decreased significantly. This alteration is associated with a decrease in beta-glucan content, which may influence cell death.

Another factor that affects the adherence of *Candida albicans* cells is that the surface of the soft lining material is considered to be a hydrophobic surface that enhances the adherence of the *Candida* species. The use of kappa-carrageenan, which is considered to be a hydrophilic material, may improve the hydrophilicity of the surface of the soft lining material, which eventually minimises the adherence of *Candida albicans* on the surface of
the heat-cured, acrylic-based soft lining material, as suggested by Yoshijima in 2010 (21).

The highly significant difference that occurred between the two experimental groups (E1 and E2) is due to the increased concentration of kappa-carrageenan powder, which resulted in an increase in its effect (22).

**Conclusion**

From the presented research, it can be concluded that kappa-carrageenan powder can be considered as powerful antifungal material and the incorporation of kappa-carrageenan powder into “heat-cure acrylic-base soft lining material” can successfully be accomplished to produce a soft lining material with antifungal properties against Candida albicans microorganisms. Also, experimental group(E2) showed a better antifungal activity when compared to the control and experimental group(E1).

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** Self-funding

**References**


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Age Related Changes of Mandible and Maxilla In Human (Morphometric Study)

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¹Department of Anatomy & Histology, College of Medicine, Tikrit University/Iraq

Abstract

The properties of bones change during life and with age, it may improving in function, but in some times function deteriorates. Throughout development and aging, bone shape alters in response to load. This study provide information on the development and normal growth of dental arches in both sex.

Forty adult person of both sex were used. They divided in to 4 groups (A, B, C, and D). We will use different size of trays and take alginate impression material to get anatomical impression for the upper and lower jaw from the male and female and obtained a dental cast which is identical to the dental arch and make a table for all measurement that acquired.

The results showed that the age have a reliable degradation effect in both sex specially after 40 years old and except in the transverse measurement of female in mandible which started to decline after 30 years excluding the inter canine width which decrease after 40 years.

Keywords: human; Morphometric study ; Age Related Changes

Introduction

The skeletal system in the body consist of ligaments, cartilages, bones and other tissues that carry out critical roles for the human body. Osseous or bone tissue, is a dense, hard connective tissue that form the internal support structure of the body and shapes the majority of the adult skeleton. Osseous tissue are not a static tissue require to be regularly maintained and remodeled. The bone mechanism are sustained in a balance to resist crack whereas optimizing the skeleton weight. As we age, the bones density initiates to reduce in both sex. This loss in density goes faster in women after menopause. Accordingly, in old age bones become more likely to break and delicate. About age 30, most people get to their peak bone mass. For a while following reaching peak bone mass, your body substitutes about as much as it loses. However less bone is replaced around age 40 and this causes the bones to be converted into weaker and thinner, increasing the risk for osteoporosis.

In response to load throughout development and aging, the shape alters. As the shape changes (narrowing walls, shifting the center of gravity and lengthening), the functional capability may also be modified, the size of cortical bone, volume of trabecular bone and bone strength turn down with age. There is some discuss in humans as to whether the patterns of formation and resorption are comparable in both gender. It must be distinguished, however, in men the age-related loss is chiefly due to thinning of the individual struts, while the loss in women is due to a diminish in connectivity.

Material and Method

40 normal healthy person with normal occlusions from both sex (male & female), with age ranging from 20-60 years were taken from normal patient in dentistry clinic in Salahddin during period extending from October 2018 to April 2019. Patients were chosen with normal Class I occlusion and they don’t have any discrepancy or disharmony in the arrangement of their teeth.

Those patient were divided in to 4 groups according to age and each group was composed of ten adults (5 male and 5 female) as follows: G1(20-29), G2(30-39), G3(40-49) and G4(50-59) year.

In response to load throughout development and aging, the shape alters. As the shape changes (narrowing walls, shifting the center of gravity and lengthening), the functional capability may also be modified, the size of cortical bone, volume of trabecular bone and bone strength turn down with age. There is some discuss in humans as to whether the patterns of formation and resorption are comparable in both gender. It must be distinguished, however, in men the age-related loss is chiefly due to thinning of the individual struts, while the loss in women is due to a diminish in connectivity.

The stock tray is chosen for impressions. Alginate possess the qualities of excellent surface detail and faster response at higher temperatures. A dental impression is an imprint of the teeth and mouth from
which shaped items can be created (11), and after pouring the impression by stone a dental cast were made. The following measurement were made for the upper and lower dental cast to illustrate the changes that occurred in dental arch:

A. Transversal measurement:
Which include
- Inter central incisor width (I1-I1)
- Inter lateral incisor width (I2-I2)
- Inter canine width (C-C)
- Inter first premolar width \{DM1(PM1)-DM1(PM1)\}
- Inter second premolar width \{DM2(PM2)-DM2(PM2)\}
- Inter first molar width (M1-M1)

B. Anterior-posterior measurement:
Which obtained by
- Incisor-canine distance (I1-C): the line between the most mesial end on the central incisor and the most distal end on the canine.
- Canine-first molar distance (C-M1): the distances between the most mesial spot on the canine and the most distal spot on the 1st molar.
- Incisor-first molar distance (I1-M1): the distances between the mesial dot on the central incisor and the distal dot on the 1st molar.
- Total arch length (TAL): the total of the spaces between the most mesial spot on the canine and the most distal spot on the 1st molar on both sides.
- Dental arch length (DAL): the area between the line of inter molar distance and the labial surface of the most prominent central incisor.

C. Vertical measurement:
Which mean palatal deepness of maxilla (PD)

We made the transversal and anterior-posterior measurements as definite by Foster et al (12). Transversal measurements were obtained by calculating the means of the biggest and smallest distances between right and left identical teeth of the jaws. Anterior-posterior measurement were made only on left side. Palatal depth and dental arch length and their length above were taken by electronic digital caliper 100 (4").

Figure (1): Transversal measurements were measured amid analogous teeth on each side of the dental arch. The width was calculated for each pair of teeth, as the mean of the maximum and minimum distances between them (A+B/2).

Figure (2): Dimensions of total arch length, Dental arch length, Incisor-canine, Canine-first molar, Incisor-first molar.

Result

The analysis including the standard deviation values and their means shown in table 1. There is no significant differences in the result of $t$ test which shown that (p>0.05) between males and females, except the inter canine width in the G1 (20-29 years) which have a significant difference (p<0.01), inter first premolar width in G2 (30-39) which have (P<0.05) and in G4 (50-59) there is difference in significant which was (P<0.05). The inter second premolar width also revealed a significantly difference of (P<0.05). The statistical analysis of male in four age groups revealed that the transversal measurement of dental arch enlarge starting from Group 1 to Group 2 but in G3 there is general decrease in all measurement of dental arch. While in female the statistical analysis shown that in G3 the all measurement began to decreased except in the inter first premolar and inter second premolar which start to decreased in the G2 (30-39) and this revealed in table (1).

As can be seen in table 2 the all anterior-posterior and vertical measurement of male in maxilla they exhibited significant increase from G1 to G2 but the G3 revealed a significant decrease in all above dimensions, while in female also there is significant increase in G1 to G2 and decrease in G3 except the incisor-first molar distance and total arch length there is a significant increase with aging from G1 to G4. Furthermore the palatal depth (PD)
show significant decrease in G4 in female while in male began in G3. Also from table 2 we can see there is a significant difference in the G2 and G3 between both sex of (P<0.05) . A significant difference of (P<0.05) also seen in G2(30-39) in incisor-first molar and total arch length.

Measurement of table 3 exposed that in female revealed a universal decrease of all measurement in G2 except the inter-canine distance that start to decrease in G3 as in male . Also a comparison between male and female in the table three, which shown no difference of significantly between them excluding the inter first premolar in Group 2 and Group 4 which is (p<0.05), and inter second premolar in Group 2, Group 3, Group 4 of (p<0.05) in addition the inter first molar in Group 2.

The statistical analysis of table 4, show that the male in G3 have a significant decrease in incisor-canine and canine first molar distance and the all anterio-posterior measurement show the decrease from G2, other than in female there is an increase from G1 to G2 and the decrease started in G3.

There is no significantly difference between male and female in anterio-posterior measurement of dental arch except the canine first molar distance and the dental arch length illustrate a significantly difference (p<0.05*) in G1.

**Discussion**

Morphometric result in the dental arch including transverse, anterio-posterior and vertical depth measurement agreed in a large degree with many others authors. Current research investigate the arch length, arch circumference, inter-molar, inter-premolar, inter-canine distances, width of all teeth and arch form on dental cast from several orthodontic centers across Jeddah city in the western region of Saudi Arabia between April 2015 and May 2016 illustrate the arch width measurements were significantly greater in male subjects than in female subjects. This result supports the findings of several previous studies, Bishara et al\(^{(13)}\) which agreed with our finding except the inter canine width of mandible that greater in female in the G1 and G3 than the male and dental arch length in G3\(^{(14)}\).

A significant bone resorption with loss of projection of the maxilla in young and old patients demonstrated when Pessa\(^{(15)}\) measured the maxillary angle (superior-to-inferior maxilla at the articulation of the inferior maxillary wing and alveolar arch) . A significant reduction of the maxillary angle with aging noted by Shaw and Kahn\(^{(16)}\) and this approve our study about the age changing of maxilla which increase in G1 and G2 but the resorption start in G3 and in female some measurement degenerate in G2.

The crowded of front teeth among the elderly caused by human jaw shrinks with age in a new study has found. Plaster molds prepared for the jaws of dental students in 1949 when the individuals were in their 20s, and record jaw molds of the same people made in 1959 and 1989 which done by swedish researchers “We found that over these 40 years there was less and less room for teeth in the jaw,” said in a university news release by Lars Bondemark, a professor of orthodontics at Malmo University. A few millimeters of shrinkage in both the length and width of the jaws, primarily the lower jaw occurred and this due to reduction in the amount of space for front teeth. Hereditary and anatomical factors affect the amount of jaw shrinkage which varies between individuals\(^{(17)}\), this agreed with our study in which all the measurement increase from age 20 and 30 and started to decrease in 40 years except transverse measurement in female which started to decrease in 30 years.

**Table (1).** Mean and standard deviation in maxilla for transversal measurement in each gender for four age groups in millimeters(mm).

<table>
<thead>
<tr>
<th></th>
<th>sex</th>
<th>G1 20-29 Mean±SD</th>
<th>G2 30-39 Mean±SD</th>
<th>G3 40-49 Mean±SD</th>
<th>G4 50-59 Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>I1-I1</td>
<td>M</td>
<td>11.934±0.834*</td>
<td>12.250±0.774 ns</td>
<td>11.63±1.11 ns</td>
<td>12.33±1.15 ns</td>
</tr>
</tbody>
</table>


Table (2) Mean and standard deviation of maxilla for Anterio-posterior & Vertical measurement for each gender in four age groups (in millimeters).

<table>
<thead>
<tr>
<th>Sex</th>
<th>G1 20-29 Mean+SD</th>
<th>G2 30-39 Mean+SD</th>
<th>G3 40-49 Mean+SD</th>
<th>G4 50-59 Mean+SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>10.44 ± 1.20</td>
<td>11.688 ± 0.774</td>
<td>11.417 ± 0.382</td>
<td>11.75 ± 1.06</td>
</tr>
<tr>
<td>M</td>
<td>22.625 ± 0.968 ns</td>
<td>23.50 ± 1.63 ns</td>
<td>20.13 ± 2.93 ns</td>
<td>22.92 ± 2.53 ns</td>
</tr>
<tr>
<td></td>
<td>21.688 ± 0.315</td>
<td>22.25 ± 2.06</td>
<td>21.417 ± 0.804</td>
<td>23.13 ± 1.24</td>
</tr>
<tr>
<td>C-C</td>
<td>M 30.438** ± 0.515</td>
<td>31.70 ± 2.42 ns</td>
<td>29.50 ± 3.24 ns</td>
<td>31.08 ± 2.90 ns</td>
</tr>
<tr>
<td></td>
<td>F 26.688 ± 0.239</td>
<td>29.63 ± 1.11</td>
<td>29.000 ± 0.866</td>
<td>30.50 ± 1.41</td>
</tr>
<tr>
<td>PM1-PM1</td>
<td>M 35.61 ± 1.48 ns</td>
<td>37.35* ± 2.53</td>
<td>35.75 ± 3.80 ns</td>
<td>37.25* ± 1.09</td>
</tr>
<tr>
<td></td>
<td>F 34.938 ± 0.826</td>
<td>34.19 ± 1.07</td>
<td>33.917 ± 0.629</td>
<td>34.50 ± 1.41</td>
</tr>
<tr>
<td>PM2-PM2</td>
<td>M 39.94 ± 2.66 ns</td>
<td>41.85 ± 1.41 ns</td>
<td>40.38 ± 4.66 ns</td>
<td>40.00* ± 1.73</td>
</tr>
<tr>
<td></td>
<td>F 39.625 ± 0.968</td>
<td>39.13 ± 2.50</td>
<td>38.83 ± 1.53</td>
<td>42.500 ± 0.707</td>
</tr>
<tr>
<td>M1-M1</td>
<td>M 43.13 ± 2.15 ns</td>
<td>45.95 ± 3.97 ns</td>
<td>43.75 ± 4.21 ns</td>
<td>46.00 ± 4.50 ns</td>
</tr>
<tr>
<td></td>
<td>F 43.38 ± 1.74</td>
<td>44.13 ± 2.50</td>
<td>43.00 ± 1.73</td>
<td>47.00 ± 1.41</td>
</tr>
</tbody>
</table>

n= (P>0.05)
* = (P<0.05)
**= (P<0.01)
**Table (2) Mean and standard deviation of maxilla for Antero-posterior & Vertical measurement for each gender in four age groups (in millimeters).**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>G1 20-29 Mean+SD</th>
<th>G2 30-39 Mean+SD</th>
<th>G3 40-49 Mean+SD</th>
<th>G4 50-59 Mean+SD</th>
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</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>M</td>
<td>31.25 ± 1.26 ns</td>
<td>30.38 ± 1.11 ns</td>
<td>29.33 ± 6.43*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>31.125 ± 0.629</td>
<td>29.33 ± 1.15</td>
<td>22.250 ± 0.354</td>
<td></td>
</tr>
<tr>
<td>G2</td>
<td>M</td>
<td>46.00 ± 2.24 *</td>
<td>41.63 ± 1.80 ns</td>
<td>42.33 ± 5.51 ns</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>42.75 ± 1.50</td>
<td>43.00 ± 6.56</td>
<td>44.500 ± 0.707</td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td>M</td>
<td>45.70 ± 1.60 *</td>
<td>41.19 ± 2.32 ns</td>
<td>41.68 ± 3.48 ns</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>42.75 ± 1.94</td>
<td>43.75 ± 6.14</td>
<td>44.05 ± 4.36</td>
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</tr>
<tr>
<td>G4</td>
<td>M</td>
<td>35.20 ± 1.92 ns</td>
<td>32.00 ± 2.16 ns</td>
<td>33.00 ± 2.00 ns</td>
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<tr>
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<td>F</td>
<td>33.75 ± 1.26</td>
<td>33.00 ± 2.65</td>
<td>31.500 ± 0.707</td>
<td></td>
</tr>
<tr>
<td>PD</td>
<td>M</td>
<td>21.75 ± 1.258</td>
<td>21.0 ± 3.61</td>
<td>22.67 ± 0.577±</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>18.25 ± 1.258</td>
<td>20.0 ± 2.65</td>
<td>19.0 ± 1.41</td>
<td></td>
</tr>
</tbody>
</table>

**Table (3) Mean and standard deviation of mandible for transversal measurement in each gender in four age groups in millimeters.**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>G1 20-29 Mean+SD</th>
<th>G2 30-39 Mean+SD</th>
<th>G3 40-49 Mean+SD</th>
<th>G4 50-59 Mean+SD</th>
</tr>
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<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>I1-I1</td>
<td>M</td>
<td>7.188 ± 0.375 ns</td>
<td>7.438 ± 0.515 ns</td>
<td>7.125 ± 0.250 ns</td>
<td>7.000 ± 0.500 ns</td>
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</tbody>
</table>
**Cont... Table (3) Mean and standard deviation of mandible for transversal measurement in each gender in four age groups in millimeters (mm).**

<p>| | | | | |</p>
<table>
<thead>
<tr>
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<tr>
<td></td>
<td>F</td>
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<tr>
<td></td>
<td>9.08</td>
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<tr>
<td></td>
<td>+ 3.84</td>
<td>+ 0.612</td>
<td>+ 0.520</td>
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<td>M</td>
<td>15.688</td>
<td>16.250</td>
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<tr>
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<td>+0.554 ns</td>
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<td>+ 0.515 ns</td>
<td>+ 1.64 ns</td>
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<td>16.08</td>
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<td>+1.23</td>
<td>+1.55</td>
<td>+ 1.38</td>
<td>+ 1.06</td>
</tr>
<tr>
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<td>M</td>
<td>24.19</td>
<td>25.88</td>
<td>25.83</td>
</tr>
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<td>+2.21 ns</td>
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<td>25.250</td>
<td>25.50</td>
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<td>+0.661</td>
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</tr>
<tr>
<td>PM1-PM1</td>
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<td>32.50</td>
<td>34.40</td>
<td>35.33</td>
</tr>
<tr>
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<td>+ 1.95 *</td>
<td>+ 2.90 ns</td>
<td>+ 3.33 *</td>
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<td>+ 2.63 *</td>
<td>+ 2.36 *</td>
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<td>37.67</td>
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<td>+ 2.39</td>
<td>+ 2.75</td>
<td>+ 0.707</td>
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<td>46.38</td>
<td>47.50</td>
</tr>
<tr>
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<td>+ 3.50</td>
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<td>+ 2.08</td>
<td>+ 2.12</td>
<td>+ 2.31</td>
<td>+ 1.06</td>
</tr>
</tbody>
</table>

n=(P>0.05)

*= (P<0.05)

**= (P<0.01)
Table (4): Mean and standard deviation of mandible for Anterio-posterior measurement for each gender in four age groups in millimeters (mm).

<table>
<thead>
<tr>
<th></th>
<th>Mean+SD</th>
<th>Mean+SD</th>
<th>Mean+SD</th>
<th>Mean+SD</th>
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<td></td>
<td>20-29</td>
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<td>50-59</td>
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<tr>
<td>sex</td>
<td></td>
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</tr>
<tr>
<td>I1-C</td>
<td>M</td>
<td>17.00 +0.816 ns</td>
<td>17.00 +0.816 ns</td>
<td>16.00 +0.816 ns</td>
</tr>
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<td>17.00 +0.707</td>
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</tr>
<tr>
<td>C-M1</td>
<td>M</td>
<td>32.75 +1.50 ns</td>
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<td>32.25 +2.87 ns</td>
</tr>
<tr>
<td></td>
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<td>31.333 + 0.577</td>
<td>31.40 +0.548</td>
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</tr>
<tr>
<td>I1-M1</td>
<td>M</td>
<td>42.25 +1.26 *</td>
<td>41.25 +2.22 ns</td>
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</tr>
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</tr>
<tr>
<td>TAL</td>
<td>M</td>
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<td>41.25 +2.18 ns</td>
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<tr>
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</table>

n= (P>0.05)

* = (P<0.05)

**= (P<0.01)
Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1. Lindsay M. Biga, Sierra Dawson, et al. Bone Tissue and the Skeletal System. Chapter 6
Study of the Course of the Inferior Dental Canal in the Iraqis Population Using Cone Beam Computed Tomography

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²Department of Periodontology, Collage of Dentistry, University of Tikrit/Iraq

Abstract

Introduction: The inferior alveolar nerve (IAN) is one of the most important vital structures found in the mandible, knowing the precise location of the mandibula canal is helpful to decrease the incidence and avoid the injury to the (MC).

Material & Method: 30 males & 30 females were selected from the data of the (CBCT) stored in the computer joined to the device and the measurements were taken as follow “ The first section in transaxial view after mental foramen, where the loop of mandibular canal is formed, was selected as point A and interludes of 10 mm were selected for subsequent measurements (respectively, points B,C,D). On these sections, the shortest linear distances (in mm) from the most lingual aspect of the canal to the outer lingual cortical plate of the mandible(L) and from the mandibular canal to the inferior border of mandibular body(I) were measured. Furthermore, the minimum distance between the buccal cortices and the mandibular canal(B) were evaluated” as in (Fig. 1). Using SPSS software Version 15 the data that had been obtained was analyzed.

Results: The results had been showed that there is no significant difference in measurements were taken between males and females groups in the measurement of the distance of the inferior dental canal to the buccal cortical and in the measurement of the distance of the inferior dental canal to lingual cortical bone of the mandible, only in the measurement of the (IDC) to inferior border of the mandible there was a significant difference in only one point

Conclusion: It can be concluded from this study that the information can be obtained by the (CBCT) about the location of the (MC) can helpful for the surgeons to avoid damage to (MC) throughout surgical procedures in the mandible, and those information can give good overview and conception for course of the (MC) through the mandible.

Key words: inferior alveolar canal, cone beam computed tomography

Introduction

The inferior alveolar nerve (IAN) is one of the most important vital structures found in the lower jaw, it is the largest branch of the mandibular nerve which is the 3rd division of the trigeminal nerve, the (IAN) has a great attention in the surgical treatment of the area of the mandible, the inferior dental canal (IDC) changes the buccolinguinal direction and the vertical direction in its course inside the mandible, it is very important to locate the precise location of the mandibular canal (MC) to make the treatment plan for the surgery would be involving the mandible and avoid the injury of the (MC) throughout the surgery. The difference in the anatomical location of the (MC) must be constantly taken into consideration to avoid (MC) injury throughout surgical procedure involving the mandible such as dental implant, extraction of impacted 3d molar, osteotomy, repair of fractured mandible, placemen of screw, and orthognathic surgery. During dental implant procedures and orthognathic surgeries it was noticed that the average of neurosensory disturbance of (IDC) was up to 77.8 and 65.1% respectively, throughout the surgical extraction of the impacted mandibular third
molar the rate of (IDC) injury was recorded to be 0.4-13.4%\(^5\), studies showed that during the bilateral sagittal split osteotomy of the mandible as the (MC) closer to the buccal aspect of the mandible the rate of (MC) injury would be increased\(^6-10\).

using cone beam computed tomography in the evaluation of the course and anatomic location of the mandibular canal was carried out in previous studies\(^{11-13}\). The aims of this study were to evaluate if the difference in the gender would be effective in the prediction of the relative location of the canal and to assess the relation of the location of the (MC) to the cortices of the mandible utilizing CBCT.

**Material and Method**

The study was approved by the Ethical Committee of Collage of Dentistry, University of Tikrit, the study was done in the department of radiology in the specialized dental center, ministry of the health in Tikrit city, from the pool of data of the cone beam computed tomography (care stream) made in Germany, 30 males and 30 females were selected without any pathological condition or fracture can affect the normal position of the Inferior Dental Canal, after making reconstructed panorama to reveal the course (IDC) from the mental foramen to the mandibular foramen in slice thickness 0.5 mm , according to the study was done by Hooman Khorshidi et al in 2017\(^{13}\) the measurement were taken as follow “ The first section in trans-axial view after mental foramen, where the loop of mandibular canal is formed, was selected as point A and interludes of 10 mm were selected for subsequent measurements (respectively, points B,C,D). On these sections, the shortest linear distances (in mm) from the most lingual aspect of the canal to the outer lingual cortical plate of the mandible(L) and from the mandibular canal to the inferior border of mandibular body(I) were measured. Furthermore, the minimum distance between the buccal cortices and the mandibular canal(B) were evaluated” as in (Fig. 1). Using SPSS software Version 15 the data that had been obtained was analyzed.

**Results**

The results had been showed that there is no significant difference in measurements were taken between males and females groups in the measurement of the distance of the inferior dental canal to the buccal cortical and in the measurement of the distance of the inferior dental canal to lingual cortical bone of the mandible , only in the measurement of the (IDC) to inferior border of the mandible there was a significant difference in only one point as the table (1) and the abbreviation were used in the table as follow:

A: the 1\(^{st}\) point
BA: the distance of the (IND) to the buccal cortical pate in the point A
LA: the distance of the (IND) to the lingual cortical pate in the point A
IA: the distance of the (IND) to inferior border of the mandible in the point A

B: the 2\(^{nd}\) point
BB: the distance of the (IND) to the buccal cortical pate in the point B
LB: the distance of the (IND) to the lingual cortical pate in the point B
IB: the distance of the (IND) to inferior border of
the mandible in the point B

C: the 3rd point

BC: the distance of the (IND) to the buccal cortical plate in the point C

LC: the distance of the (IND) to the lingual cortical plate in the point C

IC: the distance of the (IND) to inferior border of the mandible in the point C

D: the 4th point

BD: the distance of the (IND) to the buccal cortical plate in the point D

LD: the distance of the (IND) to the lingual cortical plate in the point D

ID: the distance of the (IND) to inferior border of the mandible in the point D

Table -1- the measurements of the distances of the inferior dental canal to buccal and lingual cortical plates and to the inferior border.

<table>
<thead>
<tr>
<th>Measured distance</th>
<th>Male</th>
<th></th>
<th></th>
<th></th>
<th>Female</th>
<th></th>
<th></th>
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<tr>
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<td>Mean</td>
<td>SD</td>
<td>Min.</td>
<td>Max.</td>
<td>Mean</td>
<td>SD</td>
<td>Min.</td>
<td>Max.</td>
<td></td>
</tr>
<tr>
<td>A</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>BA</td>
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<td>0.91324</td>
<td>2.50</td>
<td>5.70</td>
<td>3.8700</td>
<td>0.91324</td>
<td>2.50</td>
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<td>LA</td>
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<td>1.10694</td>
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<td>7.20</td>
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<td>1.10694</td>
<td>3.70</td>
<td>7.20</td>
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<tr>
<td>IA</td>
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<td>1.61920</td>
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<td>13.10</td>
<td>2.8100</td>
<td>11.0300</td>
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<td>B</td>
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<td>1.27817</td>
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<td>1.00</td>
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<td>2.8100</td>
<td>1.18589</td>
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<td>2.19414</td>
<td>2.10</td>
<td>9.50</td>
<td>1.00</td>
</tr>
</tbody>
</table>
Discussion

Several studies have been concluded that the knowledge of the relative position of the (IDC) to the buccal and lingual cortical bone of the mandible and to the inferior border of the mandible is important to predict and avoid the loss of sensory in the mandibular nerve\(^{(7,9)}\), the textbooks of anatomy do not describe in detail the course of the mandibular canal, there are several studies to give comprehensive understanding of the course of the mandibular canal such as clinical observation throughout surgical procedures\(^{(14)}\), measurement on dry skull\(^{(2,15-17)}\), traditional radiograph\(^{(6,18-20)}\), and computed tomography scan\(^{(7,8,21-24)}\), each one of the methods mentioned above has limitation such as change in dimension in dry skull, 2D and change in the size in the traditional radiograph, and high dose of radiation in CT scan, Kamburoğ˘lu et al. and Eizenbud et al. showed that the method of the using of CBCT to predict the course of the mandibular canal is the most non invasive and precise method used till now \(^{(25,26)}\), the results of this study have been showed there is no significant difference in the buccolingual relative position of the mandibular canal between males & females groups and those come in accordance with the studies of Simonton JD, et al\(^{(27)}\), Kane AA, et al., Angel et al\(^{(28)}\). The studies of de Oliveira Ju´nior et al\(^{(29)}\) al. and Hooman Khorshidi et al\(^{(13)}\) showed that the all measurements were smaller in female group than males group in our study that com in agreement only in measurement of mandibular canal to inferior border of the mandible and only in one point of the measurements and in the other 3 points there were no significant difference and may be difference in the races was the reason, the whole results were fairly constant in both males and females groups, our results have been suggested that as the mandibular canal runs from the entrance of the mandible in the mandibular foramen to the mental foramen the (MC) would be toward the buccal side as the (MC) runs anteriorly and the values of the means of the lingual measurements decreased as (MC) runs anteriorly and that is come in agreement with the studies of Ylikontiola L\(^{(6)}\), and Hooman Khorshidi et al\(^{(13)}\), de Oliveira Ju´nior MR. et al\(^{(29)}\).

It can be concluded from this study that the information can be obtained by the (CBCT) about the location of the (MC) can helpful for the surgeons to avoid damage to (MC) throughout surgical procedures in the mandible, and those information can give good overview and conception for course of the (MC) through the mandible.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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References


Correlation of Urine Leukocytes and Urine Bacteria Using Current Sitometry Using Urine in Patients of Children Channel Infection

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Abstract

Background: Urinary tract infection (UTI) in children is a common illness. The gold standard for UTI diagnosis is urine culture, but it takes a long time and often gives negative results. Currently developed a method of flow cytometry for checking urine sediments. This tool is able to detect leukocytes urine and urine bacteria in quick time. The purpose of this study is to analyze the relationship of urine leukocyte count and the number of urine bacteria using flow cytometry with urine culture in patients with UTI.

Method: Eighty-four patients were enrolled in this study that consisting of 43 patients with suspected UTI and 43 patients with non-ISK treated at Inpatient Installation, Emergency Installation Department of Health Sciences at Dr. Soetomo General Hospital, Surabaya for 4 months. The examination of urine leukocytes and urine bacteria was using current cytometry method (UF-500i, Sysmex, Japan). While, examination of urine culture using blood agar plate and Mac. Conkey.

Results: There was a significant relationship between the number of leukocytes and the number of colonies. The relationship between leukocytes and the number of colonies was weak, this was indicated by Spearman correlation coefficient (rho) value of 0.376. There was a significant relationship between bacteria and the number of colonies. p <0.05. The relationship between the number of urine bacteria and the number of colonies was quite strong indicated by rho = 0.729.

Conclusion: There was a relationship between urine leukocyte count and the number of urine bacteria using flow cytometry with urine culture.

Keywords: Urine leukocytes, Urine bacteria, Urine culture, Urinary tract infections.

Introduction

Urinary tract infection (UTI) in children is a common illness that found in addition to upper respiratory tract infections and diarrhea and the second most common cause of fever after upper respiratory tract infection ¹. Urine culture examination takes a long time and can give false negative results. It may delay the diagnosis of UTI, which in the end the management of UTI is also delayed, which may lead to poor UTI prognosis. The incidence of UTI in children at Indonesia ranges from 0.1% to 1.9% of all cases that children treated within 5 years (1984-1989) ².

The high incidence of UTI causes many requests for urine culture examination³,⁴. Positive urine culture when bacterial growth of more than 100,000 colony forming units (CFU)/mL from one pathogen⁵. 60-80% urine culture examination did not contain any infectious bacteria or just contaminants that can be solved by filter test ⁴,⁶,⁷. Examination of bacteria with Gram staining (sensitivity 96%, specificity 93%), examination
using 10 cell/μL hemositometer (83% sensitivity and specificity) ⁸. Microscopic examination can give results faster than culture, more simple and inexpensive. But the results of the examination require skilled, trained, and interpretation among interrogators can be different ⁶. In addition to microscopically, examination of urine and urine leukocyte bacteria can use the method of flow cytometry. Current cytometry methods can distinguish particles in urine and quantitatively calculate them, including leukocytes, erythrocytes, epithelium, bacteria, fungi, cylinders, and crystals. Sysmex UF-500i is one example of urine sediment examination tool using current cytometry method. This tool is able to check large quantities of samples with fast time ⁶,⁹,¹⁰

The sensitivity and specificity of the examination using flow cytometry to confirm the diagnosis of UTI is non-existent. Previous studies have suggested that the 65-bit bacteria/mL and 100 leukocyte/mL cutoff points gave 98% sensitivity, specificity 62.1%, negative predictive value 98.7%, positive predictive value 53.7% ¹¹. Other studies also stated that at the point of cutting of 230 bacteria/μL, urine culture <10⁵ CFU/mL obtained 95% sensitivity and 80% specificity and able to reduce the number of urine culture examination by 52% ⁹. Research using flow cytometry in Indonesia is still rare. In this research will be examined using flow cytometry to assess the relationship of urine and urine leukocytes with urine culture results.

**Method**

This research was an observational analytic research with the cross-sectional design. The research started from the literature search, research proposal preparation, sample collection and workmanship, data management, and research report preparation conducted from March until June 2013. The research was conducted at Inpatient Installation, Outpatient Installation and Emergency Installation of Department Child Health Science Dr.Soetomo General Hospital/Faculty of Medicine Universitas Airlangga Surabaya, as the place of sampling. Sample examination was performed at the Clinical Pathology Installation of Dr.Soetomo/Faculty of Medicine Universitas Airlangga, Surabaya. The sample of the study was the pediatric patient and divided into two groups: groups of patients with UTI and Non-UTI that meet the inclusion criteria.

The inclusion criteria for samples meeting the criteria in this study included patients with suspected UTI aged 2 to 18 years, patients receiving antibiotics, doing urinalysis examination and obtaining positive esterase leukocyte, positive nitrite, uric microstatic leukocyte>5/μLpb or found urine bacteria microscopic, and willing to sign informed consent. Urinalysis results obtained negative nitrite, leukocyte esterase negative, leukocyte urine microscopic <5Lpb or not found microscopic urine bacteria.

This study was started by selecting the sample according to the inclusion criteria, then the urine sampling was performed by catheterization in children less than 6 years old and the way of urine transmit clean in children more than 6 years old. After the urine sample is directly sent to the Installation of Clinical Pathology Dr.Soetomo General Hospital for urine examination using flow cytometry (UF 500i) (Sysmex Corporation, Japan) and urine culture was done within ≤ 1 hour.

All collected data collected in the data collection sheet was presented in tabular form, the diagram was processed statistically in the form of descriptive analysis. To calculate sensitivity, specificity, positive predictive value, negative and ROC curve, and to know the relation of urine leukocyte count and urine bacteria, statistic analysis test using Pearson correlation test if the data was normally distributed if the data not normally distributed using Spearman correlation test.

**Results**

<table>
<thead>
<tr>
<th>Tilt picture</th>
<th>n</th>
<th>Right</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tilt 1</td>
<td>3</td>
<td>2</td>
<td>66.7</td>
</tr>
<tr>
<td>Tilt 2</td>
<td>15</td>
<td>14</td>
<td>93.3</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>16</td>
<td>88.9</td>
</tr>
</tbody>
</table>

Table 1. The suitability between the tilt image on the flow cytometry and the bacterial form of the urine culture
Table 2: Table 2 x x2 examination of urine leukocyte count and amount of urine bacteria using flow cytometry

<table>
<thead>
<tr>
<th>Urine Culture ≥ 5 x 10^4 CFU/mL</th>
<th>UTI</th>
<th>Non-UTI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine Leukocytes ≥ 40/μL</td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td>Urine bacteria ≥ 125/μL</td>
<td>18</td>
<td>8</td>
</tr>
</tbody>
</table>

Table 3: The diagnostic value of urine leukocyte examination using urine flow cytometry

<table>
<thead>
<tr>
<th></th>
<th>Urine Leukocytes</th>
<th>Urine Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnostic value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnostic sensitivity (%)</td>
<td>65.4%</td>
<td>44.4 – 82.1</td>
</tr>
<tr>
<td>Diagnostic specificity (%)</td>
<td>81.7%</td>
<td>69.1 – 90.1</td>
</tr>
<tr>
<td>Positive predictive value (%)</td>
<td>60.7%</td>
<td>40.7 – 77.9</td>
</tr>
<tr>
<td>Negative predictive value (%)</td>
<td>84.5%</td>
<td>72.1 – 92.2</td>
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<tr>
<td>Possibly positive ratio</td>
<td>3.6</td>
<td>1.9 – 6.5</td>
</tr>
<tr>
<td>Possibly negative ratio</td>
<td>0.4</td>
<td>0.2 – 0.7</td>
</tr>
</tbody>
</table>

The results of the examination of the number of urine bacteria obtained the lowest value of 0.0/μL and the highest 58017.8/μL. Median examination results of urinary bacterial counts in the UTI group of 671,85/μL with a range of 19.4/μL to 58017.8/μL. Median examination results of urinary bacterial counts in the non-UTI group were 7.2/μL with a range of 0.0/μL to 832/μL. There was a significant difference in the number of urine bacteria between group UTI groups rather than UTI, p <0.05 (Figure.4B). Current cytometric examination can also display a scattergram image that can be viewed on scattergram B1 (Figure.3BA). Urine bacteria is represented by a purple (purple) color. There are two typical features of the slope of the bacterial scattergram namely the inclination 1 (Figure.3BB) and the slope 2 (Figure.3BC). 26 samples were tested to match the results of identification of urine cultures with current isometric illustrations. There were 18 samples that can be tested suitability and 18 samples cannot be analyzed because it does not give a typical picture. The 18 samples had bacterial counts <105 CFU/mL. The slope of 1 was 66.7% according to the culture of urine, whereas on the slope 2 there was 93.3% according to the culture of the urine (Table 1). The results of the examination of leukocyte counts with 40/μL cutoff points were 28 (32.56%) of the samples, and 58 (67.44%) of the samples were not-UTI (Table 2). The sensitivity of leukocyte examination with cutting point 40/μL to the culture of urine was 65.4%, with specificity 81.7%. The positive predictive value and negative predictive value was 60.7% and 84.5% respectively. The diagnostic value of urine leukocyte examination using flow cytometry (Table 3).
The ROC curve formed for examination of urine leukocyte counts using flow cytometry at the cutting point ≥40/μL against urine culture results ≥ 5x10^4 CFU/mL (Figure 5A). The under the curve (AUC) area was 0.793 (medium), p < 0.05. (95% CI 0.69 - 0.89). When using the cutoff point 30/μL then obtained the sensitivity of 69.2% and specificity 71.7%. The results of the examination of the number of bacteria with 125/μL intake point were 21 samples (24.4%) and 65 samples (75.6%) samples instead of UTI (Table 2). The sensitivity of examination of urine bacteria with the 125/μL cutting point for urine culture was 69.2%, with specificity 95.0%. Positive predictive values and negative predictive values were 85.7% and 87.7% (Table 3) respectively. The ROC curve formed for examination of the number of urine bacteria using flow cytometry at the point of ≥ 125/μL against the urine culture result ≥ 5x10^4 CFU/mL (Figure 5B). The under the curve (AUC) area was 0.946 (very good), p < 0.05. (95% CI 0.902 - 0.989). AUC obtained on bacterial examination is wider than in urine leukocyte examination.

**Discussion**

Based on the results obtained, there was the growth of 3 kinds of colonies of germs or more without dominant species that considered as contaminants. If there were 3 or more different organisms in urine culture then a strong allegation of shelter errors and handling of urine samples. But it can also be found in patients who use permanent urinary catheters.

There is a growth of fungi in urine culture results, these results are consistent with earlier studies, in which UTI may also be caused by pathogens (fungi, parasites, and viruses) colonizing the urinary tract. Mushroom (Candida spp) is one of the causes of UTI. The presence of fungi in urine culture is an indicator of pyelonephritis that derived from hematogenous spread (descending path). Haematogenous spread is usually as a result of bacteremia or in patients with immune system disorders.

The result of urine culture obtained significant growth. These results were different from previous studies, the difference may be due to the screening of patients suspected of strict UTI. When more rigorous screening was performed then the possibility of a positive urine culture percentage will be even more. However, it is also known that the main problem of urine culture examination lies in the collection of samples, the length of urine in the bladder, the density of the urine, if the low urine weight may be due to increased urinary frequency, the number of colonies obtained is also low. Sampling and transportation of samples, culture techniques and interpretation of results also greatly influence urine culture test results.

The incidence of UTI depends on age and sex. In this study, girls with UTI were more likely to be in the age group 0-1 years and over 5 years, while the 1-5-year-olds were more likely to suffer from boys. Girls are more at risk of developing UTI by the first year of life than boys because of different anatomical features. Moisture of the periurethral and vaginal areas stimulates the growth of uropathogenic. A shorter urethral size increases the chances of ascending infection to the urinary tract. Besides, women are more likely to receive UTI because the uropathogenic, which is part of the normal flora of the stool, can colonize in the perianal which can then rise to the vaginal introitus. The spread of uropathogenic into the periurethral, urethra and bladder can be inhibited by the normal vaginal flora (Lactobacillus sp) causing the acidic vaginal pH. If the vaginal flora is also impaired it can cause uropathogenic to stick to the urinary tract and multiply and the incidence rate of UTI will increase in estrogen deficiency.

The most common pathogenic bacteria causing UTI were Escherichia coli (E. coli). E. coli was associated with childhood UTI in developing countries. Bacteria can survive and replicate because they have virulence factors such as expression of fimbriae, synthesis of aerobactin and enterobactin (iron-binding protein), which is very high affinity to iron, useful for bacterial replication, producing hemolysin, glycocalyx-mediated adherence, somatic Ag expression, producing urease, may move and be resistant to serum bactericidal activity. There was a significant number of urine bacteria between group UTIs rather than UTIs. p < 0.05, so the number of bacteria with current cytometric examination can be used for screening examination of suspected UTI patients, thus reducing the number of urine culture requests. However, it is known that direct bacterial examination cannot distinguish between uropathogenic or contaminants.

There were two images of the slope of the scattergram bacteria (scattergram B1), which a slope of 1 that formed from densely dispersed points and extends along a diagonal line. This describes was suspected of the bacteria in the form of coccus. Tilt 2 was the slope...
formed from the points collected/concentrated in the narrow zone. This picture of the possibility of urine bacteria in the form of stems 16. The compatibility between the slope 2 and the shape of the stem bacteria was 93.3%, while for slope 1 only 66.7%. The same result was obtained in the previous research which got 2 slope suitability of 100% while the slope of 1% was 75% from 47 samples under study 16. The formation of a typical slope image is caused by the working principle of bacterial examination using a red (semiconductor) laser that concerns the bacterial distribution of B_FSC (describes particle size) and B_FLH (describes the intensity of the nucleic acid color of the particles) 14.

The result of the analysis shows that there was a significant correlation between urinary leukocyte count and the number of colonies, but the relationship was weak, it indicated by the correlation coefficient (Spearman) only 0.376. The flow of urine can usually clear the urinary tract of pathogens. Urine itself also has specific antimicrobial properties, including low urine pH, high urea content, high organic acid content, polymorphonuclear cells, and Tamm-Horsfall glycoprotein, which can inhibit bacterial adherence in the bladder mucosal wall 6,15. The relationship between urine bacteria and the number of colonies was quite strong, this indicated by the value of the correlation coefficient (Spearman) that quite large by 0.729. Urine bacteria found in current cytometric examination was associated with positive urine culture results but direct bacterial examination cannot distinguish between uropathogenic or contaminants 15.

**Conclusion**

Based on the results of research and discussion can be concluded that there was a weak relationship between urinary leukocytes and the number of bacterial colonies, but a strong relationship between urine bacteria with the number of bacterial colonies.

**Conflict of Interest**: There is no conflict interest

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**Ethical of Clearance**: This study was approved by Ethical Commision of Health Research Faculty of Medicine University of Airlangga

**References**


Visum Et Repertum and Forensic Examination of a Rape Case as a Sexual Crime Towards an Indonesian Migrant Worker in Taiwan

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Abstract

This research aims to describe the forensic examination in the handling of the sexual crime which happened to an Indonesian migrant worker in Taiwan. It is well known that the level of human rights protection in Taiwan is quite good compared that of the other destination countries for migrant workers. Yet, is the good level of human rights protection balanced also with medical treatment or medical forensic examination of the Indonesian migrant worker sexual crime victim who worked in that country. This research uses a non-doctrinal method. This is because the law is not only a normative theory. Yet, it also views the sociological aspects which happen in the society. The research results show that Taiwan is a country with a worrying rate of sexual crime. Yet, it has underwent good procedures in forensic examination, including in visum et repertum and other examinations for sexual assaults which happened to migrant worker victims. The visum et repertum preliminary examination which applies in Taiwan is done through a special unit which is provided for sexual assault victims. The results are acknowledged as evidences of sexual crime. It will be directly coordinated with the Taiwan special unit of justice for sexual crime.

Keywords: Forensic, Sexual Crime, Migrant Worker

Introduction

Sexual crime is a serious crime which causes prolonged physical or psychological trauma if it is not handled properly, not only for the victims but the effect can also befall upon the victims’ family.¹

The number of sexual crimes in Taiwan is considered high and the rate tends to increase every year. In 2017, the number of sexual crimes reported to the Taiwan women’s protection bureau was 14,217 cases, and it increased 50 cases to 14,267 cases in 2018. Although in 2018, a special movement for the protection of sexual crimes victims has been formed with the “me too” movement, which is the slogan of a global movement in the protection of sexual crimes victims, in which the Taiwanese government has actively participated in this global campaign, but it seems unable to reduce the reported number of sexual crimes cases in Taiwan.²

Sexual violence which occurs to Indonesian migrant workers in Taiwan each year is no less than 100 cases, which is usually carried out by the employers, the employer’s close relatives and their own employment brokers. During the last 6 years since 2012 – 2018, there are 633 cases of sexual violence which have befallen to Indonesian migrant workers.³

Sexual violence or crime is indeed the crime that has been the highly reported, but the number of cases that are successfully handled and processed until tried and sentenced is tiny, especially if the case happened to Indonesian women migrant workers in Taiwan.⁴

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and dirty hence they are afraid and very embarrassed to report it (5).

Forensic visum et repertum examination has an effective time span of 72 hours so that if the crime is not handled as soon as possible, namely in the golden time where the time span is 72 hours after the rape, it will be arduous to find the evidences needed (6). Since these female migrant workers usually report the assault weeks or years after it happened, hence the perpetrators rarely get caught in the law, except for one phenomenal case that has been recorded to successfully enter the court and the perpetrator was severely sentenced to 7 years and 10 months of imprisonment (7).

The perpetrator is a member of the city council in Hualien, Taiwan. He committed a violence against an Indonesian migrant worker who was employed as a nurse at his house. Even though the case was appealed to the Taiwanese Supreme Court by the perpetrator, the court of appeal and the Taiwanese Supreme Court still rejected it. (8)

Most of the sexual crime’s perpetrators escaped the law due to the lack of knowledge from the victims and the response from law enforcement officials themselves. Most perpetrators will try to eliminate the evidences by threatening the victims not to report or order them to bathe in order to remove the traces of sexual intercourse on the victim’s body, thus making it difficult for the law enforcers to carry out visum et repertum as a forensic effort, furthermore the victims who do not want to report immediately, will make the evidences disappear after 72 hours. (9)

Forensic medical science is a solution for the verification difficulty in ensnaring the sexual crimes perpetrators (10). With forensic medical science, the law that was still in the dark in finding or ensnaring the perpetrators can find a bright spot in allowing the perpetrators to be ensnared by the law. (1)

Rape is an act of violence which the sexual intercourse is not on the basis of mutual consent but it is forced. Rape is difficult to prove legally even though there may have been the evidences collection and examination, unless forensic test is carried out (11). In the Taiwanese Constitution which is the criminalization basis for rape, it is stated that “anyone who with violence or threat forces a woman without marriage relations to have sexual intercourse with him and because of his offense of rape, is sentenced with an imprisonment up to 12 years”. (12)

From here, the verification that the intercourse happened must be proven first thus to be called a rape. While to verify it, one of ways is by identifying the presence of the perpetrator’s sperm in the victim’s body through a forensic test conducted in a more specific area in her vagina. However, it needs to be understood that the sperm can only live in the vaginal cavity for a maximum of 3 days or 3x24 hours. While in a dead state, the sperm can still be found or remain in the vagina for a maximum of 7 days or 7x24 hours. (13)

It will be difficult to prove the rape if after a crime has been committed by the perpetrator, the victim then does not report it within a specific timeframe so that it becomes difficult in proving through visum et repertum (14). Not to mention if after the rape, the perpetrator forces the victim to clean the body and all the traces of rape that had occurred (15).

So in such rather complex occurrences, forensic science may help revealing the occurrence of sexual crimes and the perpetrator of said case. (16) Forensic Medicine has techniques which are commonly used in serious crimes such as murder and rape, based on automatic fingerprint recognition or search technology and automatic DNA database search or visum et repertum examination or forensic post and ante mortem which result major changes altogether on forensic medical techniques in the resolution of criminal cases. (16)

The role of Forensic Sciences in obtaining clarity for the sake of proving that a rape crime has indeed occurred is to examine the victim thoroughly in order to obtain the evidence through the examination of special forensic expert doctors for rape victims who have died and obstetricians for examination of victims. The results of the examination were written in the form of visum et repertum which would later be used as evidence in the category of letter evidence to be used in court plus the statements of the expert or doctor concerned (who conducted the forensic/visum et repertum test) to be present if necessary for questioning as an expert before a court hearing. (16)

The accuracy of a forensic doctor or forensic laboratory in analyzing victims to obtain evidence will determine the fairness which will be obtained by the victims and also the perpetrators (17). Because if there has been found any errors or misdiagnosis in the forensic laboratory analysis conducted by certain doctors or
specialists, it may result in fatal consequences such as the perpetrators may be in released or they may be arresting the wrong person who did not commit the said crime.

Method

This study is a legal normative study using literature study. It discusses the visum et repertum and forensic examination of a rape case as a sexual crime towards an Indonesian migrant worker in Taiwan.

Finding and Discussion

The steps required to find evidences of sexual crimes such as rape in Taiwan against Indonesian migrant workers is similar to the general procedures applied for the other citizens, thus there is no specific exception and discrimination in law enforcement treatment in Taiwan in this case. As rape and sexual crime cases in Taiwan has indeed become major issue which is of a serious concern of the Taiwan government itself. (9)

The stages to find evidence through a forensic examination in supporting further forensic analysis of victims of sexual crimes such as rape which befall upon migrant workers are as follows:

1. Investigating the crime scene by officer (police) stage
   - Check the victim’s condition whether it requires first aid, identify the victim’s age, and analysis of alleged sexual accident scheme
   - Identify the victim’s body condition to check whether there are signs of violence
   - The process of securing evidence and the crime scene from unauthorized persons
   - The process of collecting evidence properly such as victim’s pants and shirts, blood stains, mucus, scattered strands of hair, spots on fabric, et cetera.
   - Examine the victim’s expression
   - Examine the condition of the victim’s clothing (torn, broken, and such)
   - Sending the victim by providing the visum et repertum form to the nearest hospital and have them refrained in washing themselves, escorted by police thereby the results would be more precise.

   - If required, the victim may be isolated for the purpose of forensic examination.

2. The stage of collecting the evidences at the crime scene by the police
   - Materials which involves chemicals, drugs, and alcohol
   - Physical materials which includes clothing and cloth used to bind or lock the victim
   - Biological materials such as sperm, blood, hair and mucus/saliva

3. The early stage prior conducting visum et repertum upon the victim by a doctor
   - Mandatory written request from Taiwan prosecutors or a particular police who has been mandated
   - The victim came with special police assistance
   - There is a victim’s consent, if the victim is unconscious, the consent may be obtained from the victim’s family
   - To be examined as early as possible to avoid the destruction of evidence

4. The examination of the victim

This victim examination is divided into two parts which are general and special examination. The general examination is as follows:

   - The face, hair, and emotions of the victim
   - Signs of the victim was intoxicated, unconscious, and/or was exposed to narcotics
   - Signs of violence by examining all parts of the victim’s body thoroughly
   - Evidences which might be attached to the body of the victim who is allegedly belongs to the perpetrator
   - Identification of the perpetrator’s age
   - Identification of height and weight
   - Further identification if required
   - Special examination includes (19):
     - Genital skin, to check whether there’s rupture or other signs of violence
- Eritema vestibulum examination
- Vaginal bleeding
- Vaginal abnormalities which might result in infection
- Hymen examination
- For female victims who have had intercourse prior the incidence of rape/sexual crime, would then be sought whether there has been another rupture for the women who have never given birth
- Examination of the presence of sperm cells left in the vagina
- Anal examination under the allegation of anal rape possibility, rupture/scar might be visible
- Laboratory tests such as blood tests, sperm fluid, pregnancy tests, examination of other diseases such as the potential of contracting HIV, Gonorrhea, and hepatitis. Also an examination of body fluids or hair which might belong to the perpetrator.

5. History/interview to the victims
6. Victim’s physical examination
7. Assessment

There are two traumas which become the result of the assessment after the alleged presence of sexual crime, which are as follows:
- Non-genital trauma
- Genital Trauma

The aspect of sexual crime victim’s physical examination, based on the procedures practiced by medical forensic in Taiwan are as follows: (20)

**Table 1. The aspect of sexual crime victim’s physical examination**

<table>
<thead>
<tr>
<th>Type of Examination</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Examination of Vital Signs</td>
<td>100%</td>
</tr>
<tr>
<td>Top to toe examination</td>
<td>97%</td>
</tr>
<tr>
<td>Genital examination</td>
<td>80%</td>
</tr>
</tbody>
</table>

Result of Top to Toe examination: (21)

<table>
<thead>
<tr>
<th><strong>Table 2. The Result of Top to Toe examination</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sign of abnormality</td>
</tr>
<tr>
<td>No sign of abnormality</td>
</tr>
<tr>
<td>Rejection of Examination Procedures</td>
</tr>
</tbody>
</table>

Results of Genital Examination:

**Table 3. The Results of Genital Examination**

<table>
<thead>
<tr>
<th>Genital Examination</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wound found on the victim’s genital</td>
<td>85%</td>
</tr>
<tr>
<td>No wound found on the victim’s genital</td>
<td>15%</td>
</tr>
<tr>
<td>The victim did not carry out internal examination due to pregnancy</td>
<td>12%</td>
</tr>
<tr>
<td>Rejection of Examination</td>
<td>5%</td>
</tr>
<tr>
<td>Examination cannot be carried out due to other factors</td>
<td>3%</td>
</tr>
</tbody>
</table>

Margareth Stark states that the doctor’s description of sexual crime may become the main reference of law enforcers in examining the victim. The doctors them selves will become the reference when they are present in court as expert witnesses. (22)

What was practiced in medical forensics in the handling of sexual crime towards migrant worker are actually almost the same as that which happens in Indonesia (23). It’s just that the support of technology in medical forensics and in the examination process in Taiwan is more advanced as they are equipped with more updated technologies.

**Conclusion**

Sexual crime in Taiwan is a serious crime which attracts special attention from the government. This is because the rate of cases is high. It worries the society. Moreover, there is a tendency for the increasing rates of sexual crime, which makes it become a serious crime in that country.

Rape is a form of sexual crime, which is also a crime against the human rights. This is because it causes severe and serious effects for the victim and the family. The evidencing process is also complicated and difficult to handle. Yet, it can be done through detailed forensic
examination, supported by technological advancement in Taiwan. This eases the process of catching the perpetrators. The evidences obtained in the Taiwanese medical forensics are valid evidences in court in front of the judges from the district to the Supreme Court level. They are to decide whether the defendant is guilty or not. Forensics may show the identity of the perpetrator/defendant in a sexual crime, as well as the pattern and type, the time, the location, the modus operandi and the motives of that crime.

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**Ethical Clearence:** Yes

**Conflict of Interest:** No

**References**


Forensic and Toxicological Tests for Protecting the Patients of Esthetic Beauty Clinic as the Constitutional Right in Indonesia

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Abstract

Esthetic beauty clinic is many people’s need, but the critical point of protection for its patients is still weak. Many problems often affect the patients of esthetic beauty clinic. Patients deserve protection of medical service standard constituting something important to maintain and to improve. Forensic and toxicological medical sciences are needed to give adequate protection to the patients of esthetic beauty clinic. Through its scientific method, the state could prove, prevent, and take action all at once against the esthetic beauty clinics operated haphazardly. The clinic selling, concocting, using, and promoting hazardous and toxic drug and cosmetic can be detected and acted on, while forensic test was intended to prove the consequence or output of esthetic beauty clinic’s service. This scientific method supported the protection and the right of patients of esthetic beauty clinic, corresponding to the constitution.

Keyword: clinic; protection; forensic; toxicology; aesthetic

Introduction

A new trend of medical aesthetica, is an individual’s aiming to get healthcare treatment including both general checkup and rehabilitation; it is usually performed by the patients in developing countries looking for medical service in the developed countries in order to get high-technology service quality, as shown by the finding of research below.

In recent years, a variety of beauty clinics appear more prevalently in Indonesia, for example, Dr. Affandi’s esthetic beauty clinic, Natasha Skin Centre, London Beauty Centre, Erha, and Larissa providing beauty treatment product that can make the patients excited. Beauty Clinic is an ambulatory healthcare service provider providing medical care service (consultation, examination, treatment, and medical action) to deal with many beauty-related conditions/diseases (appearance esthetics) among individuals, conducted by medical workers (physician, dentist, specialist, and dental specialist) corresponding to their skill and authority.1

The result of researches on trend beauty in Asia today shows that:

This beauty business growth builds on women and metrosexual men’s want to appear perfectly in Indonesia, so that to achieve their want they voluntarily spend their money to buy the expensive facial and body treatment products. The wish to appear beautifully or charmingly and well-groomed is a human’s physical need, particularly to women. Even along with the times, men as if do not want to be left behind the women in the term of tidy and charming appearance; such the men are called metrosexual men. It is this reality that encourages many companies operating in Beauty and Personal Care to keep innovating to create high-quality product the patients look for in order to win the market.

Beauty and esthetic clinic is inseparable from the contribution of physician serving as consultant and the one giving treatment or taking action in the beauty clinic’s service, and the business of beauty clinic itself. It is important to study because physician is bond to
Indonesian Medical Ethical Code as the legal source in undertaking his/her profession and Indonesian Honor Chamber for Medical Discipline as the supervision of physician’s professionalism in Indonesia; therefore when a physician is suspected to break the law, he/she should be processed immediately, because knowledge and science he/she has should be used duly rather than for profit-oriented purpose only and breaking medical ethical and professional code.

The protection of patients from the service provided by esthetic beauty clinic is a distinctive problem, so the basic problem in this study is how the law protection model is for the patients of esthetic beauty clinic in the citizen’s constitutional right perspective. The form of protection provided to the patients of esthetic beauty clinic is, among others, forensic test on esthetic clinic.

Responsible of the state for fulfilling the citizens’ constitutional right to the service of Esthetic Beauty Clinic in Indonesia are public and private. The government’s responsibility has been mentioned in Article 28D, clause (1) of 1945 Constitution concerning the right to guaranty, law protection and certainty and Article 28H clause (1) related to the right to health and to get healthcare service. The rights fulfillment is accomplished using the legal instrument and the institution of state authorized in licensing, organization, building, supervision, monitoring, and evaluation in the organization of esthetic beauty clinic. The esthetic beauty clinic’s responsibility includes: fault, risk, product, and professional.

Forensics is always related to crime (unlawful deed). In books of forensic science, forensic science is generally defined as the application and the utilization of certain science for law enforcement and justice purpose. In investigating a criminal case, observation on physical evidence and interpretation on the result of analyzing (examining) evidence is the main instrument of investigation. Forensic test, in this case, examines the evidence existing, coming from, used, and sold in esthetic beauty clinic, to find out whether or not it has been appropriate or there is malpractice, default, or other finding. In addition to forensic test, there is toxicological test on cosmetics and services provided by esthetic beauty clinic to find whether or not the cosmetics contains mercury or other hazardous materials.

State or state apparatus is authorized to conduct forensic and toxicological tests on esthetic beauty clinic and to test the drugs used to find out whether or not they have been corresponding to the standard specified. The authorized one is also entitled to conduct toxicological test on the drugs sold by esthetic beauty clinic for the sake of protecting patients. Drugs or cosmetics used by esthetic beauty clinic and having obtained license from BPOM and halal certificate from MUI could undertake forensic and toxicological tests suddenly to find out whether its ingredients or contents have been corresponding to what proposed when the halal certification or BPOM license was filed or are different.

**Method**

This study was a sociological research. The analysis used in this sociological research was socio-legal approach, the law interacting with people (law in action). In addition, social (non-doctrinal) research method was also used with interactional (micro) approach using qualitative analysis or qualitative method.

**Findings and Discussion**

The factual condition occurring should be the legal relationship between physicians handling the patients in esthetic beauty clinic and the patients; it is called therapeutic relation. Juridically, therapeutic relation can be defined as a legal relation between physician and patients in esthetic beauty medical service professionally based on the competency corresponding to certain expertise and skill in medical field. Therapeutic relation builds on mutual trust, as formulated in the Preamble of Indonesian Medical Ethical Code contained in Republic of Indonesia Minister of Health’s Decree Number 434/Men.Kes/SK/X/1983 about the enactment of Indonesian Medical Ethical Code to physicians in Indonesia.

“Since the beginning of written history regarding mankind, a trust relationship has been known between two individuals: the curer and the sufferer. In modern age, the relation is called therapeutic one between physicians and patient, conducted in confidential circumstance and enclosed with any emotion, expectation, and worry of human creature”.

The problem arising later is the implementation of norms and rules of law included in many regulations, because the facts existing are sometimes different from the ideal norms, moreover if anything to be governed has distinctive characteristic and specification. Similarly, healthcare (service) has its typical characteristics, as explained below:
1) Uncertainty principle. It means that an individual does not know certainly when he/she will need healthcare service. Basically, no one wants to be sick and hospitalized; everyone to keep healthy.

2) Information asymmetry. When an individual is sick, the decision to purchase healthcare service as needed is on the physician’s hand or healthcare center (clinic or hospital). If they are told to follow a surgery procedure, patient can be said as not knowing whether or not they need the operation. Although informed consent\(^8\), has been known recently to involve patients in making decision over medical action to be done by physician in esthetic clinic, the patients remain to be not on the position knowing their health status at that time like the physician treating them.\(^1\)

Another factor also resulting in the expensive healthcare service is labor-intensive one. To provide healthcare service, multidisciplinary approach and automation production process are required.

The legal implications sometimes arise along with the relationship between healthcare service provider and service patients, the ones instead are understood very poorly by the public. For example, see Prita case or other malpractice cases. The point the people (or perhaps service provider) understand poorly is the presence of such therapeutic relation. The understanding on the emergence of legal relation in service in esthetic beauty clinic is called medical service, legal foundation of medical care service relation, legal position of parties in medical service, and risk of medical service.

A new protection model is required to protect the patients of esthetic beauty clinic. The old law protection model has been replaced with the new one. It is this reconstruction of law protection model that will be the guideline or guidance in organizing esthetic beauty clinic in Indonesia in the form of forensic test that will be prevailed periodically and randomly.

It was reported for the first time that Josep Bonaventura Orfila in a trial with toxicity trial in animals in France in the 19th century and with his toxicological book can convince the judge to remove an assumption that the toxicity-induced death is cause by mysticism.

Recently in an investigation of a criminal action, it is an imperative to apply authentication and examination of physical evidence scientifically so that the material truth can be achieved. In the presence of scientific authentication, police, district attorney, and judge should not rely on the suspect or live witness’ admittance admission for investigating and settling a case. Because live witness may lie or may be told to lie, based on the information of witness intended, the achievement of truth enforcement objective in the criminal case process intended.

In authenticating and examining scientifically, we know the terms forensics and toxicology.

Generally, forensics can be defined as the application or the utilization of certain science for law enforcement and justice purpose. Forensic medical science is one of specialist branches of medical science utilizing medical science to help law enforcement and problem solving in legal domain. Indeed originally the forensic medical science is intended only to justice purpose, but in its development it is also utilized in non-justice areas, including protection or prevention.

The scope of forensic medicine develops over times, from originally the death of crime’s victim, unexpected death, unknown corpse, to still alive victim of crime, or even biological skeleton, tissue, and material putatively coming from human being, and to concoction and its consequence to human beings.

The type of case also expands from murdering, mistreatment, sexual crime, domestic violence, child abuse and neglect, dispute in divorce, paternity testing, to human right infringement. While Forensic medical science used to deal with the dead victim is called forensic pathology, the one dealing with the live victim or the suspect is called clinical forensic medicine or called police surgeon in some states. Forensic test on esthetic beauty clinic is called clinical forensic medicine.

Toxicology itself has been known since the human civilization development in looking for food, of course having tried a variety of materials including botany, plant, and mineral. Through its experience, it identifies food safely and dangerously. Meanwhile, the word toxic is a term used to explain and to represent a variety of “chemicals” clearly hazardous to body. The word “toxic” come from Greek stem ‘tox’, meaning bow and arrow.

In which bow and arrow is used at that time as the weapon in war, the arrow of which is always toxic (poisonous). “Papyrus Ebers (1552 B.C.)” of ancient Greek contains complete information on medication and drug. Papyrus also contains toxic concoction such
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as antimony (Sb), cooper, lead, hiosiamus, opium, turpentine, and verdigris (green crust in copper surface). Meanwhile, in India (500 - 600 B.C.)

Susrata Samhita wrote about many poisons coming from food, plant, animal, and snake bite antidote. Hippocrates (460-370 B.C.) was known as toxicologist in his time. He wrote about snake poison and in his book also represents that ancient Greek has had knowledge on antidote. In his book, he groups poison from plant, animal, and mineral. It proves that toxic effect resulting from toxin has bee known by human beings since the early development of human civilization. Therefore, this toxic effect is utilized widely for murdering or suicide purpose. To prevent poisoning, people always attempt to find and to develop the attempt of preventing or offering poison (toxin).

This attempt is in line with the development of toxicology itself. The objective of toxicological test is to find out whether or not the drugs and the chemicals used in the beauty treating process contain toxin or prohibited material, its use has been consistent with the dosage expected, patients know the risk and ingredient of drugs and cosmetics in the services in esthetic beauty clinic.

Orfila designed a variety of methods to detect toxin and to show the importance of chemical analysis as the legal evidence. Orfila worked as a medicolegal expert in Sorbonne, Paris. Orfila played an important part in LaFarge (murdering case using arsenic) in Paris, using arsenic analysis method; he proved death due to arsenic poisoning. M.J.B. Orfila was known as father of modern toxicology because of his interest in the effect of toxin. In addition, he also introduced quantitative methodology into a study on toxic action on animal; this approach yields a modern toxicological area, forensic toxicology.

Simply and briefly, toxicology can be defined as a study on the essence and the mechanism of hazardous effect (toxic effect) of many chemicals on living organism and other biological systems. It can also discuss the quantitative assessment on the severity of and frequency of such effect in relation to the creature’s exposure. While chemicals are said to be toxic, most of them are defined as substance potentially exerting hazardous effect on certain biological mechanism in an organism.

Toxicity is a term usually used in comparing a chemical with others. It is common to state that a chemical is more toxic than other chemicals. Comparison is less informative, unless the statement involves information on biological mechanism discussed and in what condition the chemical is hazardous. Therefore, toxicological approach should study on the effect of many chemicals emphasizing on the mechanism of the hazardous effect of such chemical and various condition in which the hazardous effect occurs. Generally, hazardous/pharmacological effect results from the interaction between chemicals (toxin or biological active substance) and receptor.

Scholarship in forensic and toxicological medicine is the fundamental basis the authorized apparatus can use in conducting forensic test and toxicological test on esthetic beauty clinic in Indonesia. Patients’ rights can be fulfilled if the clinics evidently endangering the patient by using, storing, and selling hazardous toxic drugs and cosmetics or hazardous substance can be acted on by Health Act, and sentenced with imprisonment.

**Conclusion**

Forensic and toxicological tests are scientific methods very effective in protecting the patients of esthetic beauty clinic. The relationship between physician and patient in esthetic beauty clinic is therapeutic one based on honesty and first-rate service. The protection for the patients of beauty clinic from malpractice and physician and medical worker’s default in clinic is a constitutional right.

The esthetic beauty clinic evidently hazardous, using hazardous drug, committing malpractice or default should be acted on legally. Forensic and toxicological tests should be conducted suddenly, randomly and routinely, thereby can give security and protection for the patients of esthetic beauty clinic.

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**Ethical Clearance:** Yes

**Conflict of Interest:** No

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Principal Fairness and Equity within Healthcare Services based on BPJS Kesehatan

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Abstract

Health services are the right of all Indonesian citizens. The health service system in Indonesia refers to the class system because based on the Law on BPJS itself it explains that the Indonesian public health service is in accordance with the premiums paid. The class system often triggers differences in services that are not the same equity and risk discrimination against health services. BPJS is a Universal Health Coverage (UHC) system in Indonesia. UHC system abroad, the health service is not based on classes, premium payment is the same. Comparison to other countries is not a wise view but it can be a benchmark of the progress or value of the service system in our country. Gradually, non-class based health services must be implemented to equalize and prevent discrimination in health services so that a conducive, fair and patient-based service environment can be created.

Keywords: BPJS, fairness, universal health coverage, healthcare

Introduction

Declaration of Human Rights Article 25 explicitly stipulates that every citizen has the right to get protection if he/she reaches old age, is sick, disabled, unemployed and dies.¹ Health financing in Indonesia, only around 2.2% of the Gross Domestic Product (GDP), is very risky in administering state health with a high morbidity rate. The funding is very far compared to the WHO’s recommendation (5% of GDP/year). Moreover, it is found out that around 70% of Indonesian people pay for health services independently which can affect family/individual finances.²

WHO shows that nearly 89 countries have formed a legal unit that regulates the implementation of UHC and on average it has imposed UHC financing on the tax sectors. Such sectors include the cigarette tax and liquor tax which were indeed enhanced for one of them subsidizing national health financing in several countries.³

Who itself conducts research on UHC in general. That is because related research is caused by the uneven prevalence of advice given by UHC to recipients of health services. For example, there are many patients who have HIV-AIDS throughout the world who have not been covered for antiretroviral therapy. Aspects to be investigated by WHO include among others concerning maternal and child health, infectious diseases, services and available health systems.⁴

In the framework of organizing national health insurance that can reach all levels of society, in Indonesia Law No.24-2011 concerning the Social Security Organizing Agency (BPJS) which on January 1, 2014, began to organize social health insurance for all Indonesian. In Article 3 of Law No.24-2011 concerning BPJS, the National Social Security System aims to provide guarantees for meeting the basic needs of a decent life for each participant and/or family member.⁵

The implementation of the Social Security System (SJS) in Indonesia refers to the operationalization of Workers’ Social Security (Jamsostek) under Law No.3-1992 because it is a permanent program that is mandatory and is open to all workers. Therefore, Jamsostek was reformed into the National Social Security System (SJSN) based on Law No.40/2004. The operationalization of...
Jamsostek for >20 years is still exclusive in the sense that it has not yet reached the entire membership of workers including informal sector workers. Problems arise related to the Social Security program membership target at the end of 2013 which only reached 25% of the workforce. If the Jamsostek program is continued, there will be human rights violations that can be addressed to the Government, because of its attachment to the UN Universal Declaration of Human Right dated December 10, 1948, so that Indonesia as a UN Member needs to comply with the UN-Declaration.\(^{(6)}\)

In its implementation, the National Social Security System implements a law to guarantee the rights of all citizens in Indonesia to obtain equal health services. It is expected that with the existence of SJSN, all citizens will later register as JKN-KIS participants which incidentally aims to ease health financing for the Indonesian people in general.\(^{(6)}\)

BPJS which is categorized as social insurance applies the equity principle, which is the similarity in obtaining services in accordance with its medical needs that are not bound by the number of contributions paid. BPJS also has the authority to collect payment of participant contributions and provide administrative sanctions for participants who do not fulfill their obligations.\(^{(7)}\)

There are fundamental problems, namely the premiums that must be paid by participants are not in accordance with the counts of experts or do not match the actual counts commonly used in programs like this. This condition creates an underfunded program situation that structurally will affect the sustainability of the health insurance program. The challenges facing the implementation of the JKN-KIS program today are faced with the issue of the financial soundness of the Social Security Fund (DJS), which is experiencing a deficit due to inadequate contribution rates compared to the extent of the benefits stipulated.\(^{(8)}\)

Basically, social security is carried out in line with the principles of the welfare state (Welfare state) that is developing widely in Western European countries, the United States, Australia and New Zealand. This system is implemented as an alternative to overcome poverty and multidimensional inequality. This paper intends to compare the differences in the health care system in Indonesia with other countries and then look at the justice side of the health services received by each citizen. Later this comparison will provide input that is expected to be a reference to improve the UHC system in the future for Indonesia.

**Findings and Discussion**

In the framework of organizing national health insurance that can reach all levels of society, in Indonesia has been passed Law No.24-2011 concerning the Social Security Organizing Agency (BPJS) of Health which on January 1, 2014, began to organize social health insurance for all Indonesians, able communities or unable. In accordance with Article 3 of Law Number 24 of 2011 concerning BPJS, the National Social Security System aims to provide guarantees for meeting the basic needs of a decent life for each participant and / or family member.\(^{(9)}\)

BPJS according to the BPJS Law No.24-2011 About BPJS is a public legal entity formed to carry out social security programs that are accountable to the president. BPJS funding sources are from the government (Recipient Contribution Assistance (PBI) and participants who pay contributions. BPJS membership according to Article 16 paragraph (1) explains that all citizens (other than Employers, Workers and PBI) are required to register themselves and their family members as Participants to BPJS. So the organizer of the JKN BPJS is the government and social security program participants, including foreigners who work for a minimum of 6 (six) months in Indonesia.\(^{(9)}\)

Problems often arise in the implementation of the Health BPJS. These problems include the low premiums paid, under-standard premium calculations, differences in claims of INA-CBGs with real costs, indications of fraud by health service providers, differences in perception of coding in hospitals, BPJS claims arrears to hospitals, and BPJS socialization to the community is still quite low.\(^{(10)}\)

These various forms of problems have the risk of causing discriminatory services to the community. Coupled with the provisions of the class system, which distinguishes the amount of premium to be paid for BPJS membership every month. If we look at developed countries, one of which is Finland, the health care system is different. The Local Government Health Service System in Finland regulates almost the majority of all health services in the country.\(^{(11)}\)

This system applies to all Finnish residents who are registered as permanent residents. Every citizen has the
right to receive all types of health services desired, according to the level of specialization needed. The services provided include holistic services from promotive, preventive, curative and rehabilitative.

Figure 1. Finland’s Health Financing System

Figure 2. Health Financing System of Australia
Then the health financing system in other countries can also be used as an example, namely Australia. In Australia, the health financing system is called Medicare, which is funded by Medibank, funded by the government through state agreements. States have an obligation to maintain the health of the population included in their area. The medicare financing itself comes from the income tax of Australian residents and there is income from people who can contribute to health through(12)

Comparison of the two types of health services in developed countries implies the absence of a class system in the payment of health contributions. The absence of such a class system can also reduce discrimination in health services. The principles of equality in services that promote justice based on effective and efficient services have been listed in some regulations.

Some of these regulations are reflected in one of them the 1945 Constitution which explains explicitly that each health service must not be treated differently from one another, in accordance with article 28 I paragraph (2).(13)

In addition, Act 36 of 2009 concerning Health, articles 4-7 states that every citizen has the right to health services that are equitable, safe, affordable and the community has the right to independently determine the best health services for themselves. Article 9-13 explicitly describes the government’s obligation to fulfill equitable access to health services for the community.(14)

In-Law No.24-2011 concerning BPJS itself, it explains in article 2 that the principle of administering BPJS is to provide health care guarantees based on social justice for all Indonesian people.(15) Each country agrees and recognize the right of everyone to get health services with good standards which include: efforts to improve children’s health by reducing infant mortality; improvement of aspects of industrial health services; and comprehensive holistic management for any endemic, pandemic or other diseases that require treatment.(16)

In the Doctor’s Oath Pledge, which is listed in Government Regulation No.26-1960 Article-1, explicitly explains that a doctor must carry out the medical profession without observing differences in ethnicity, religion, race, culture and social position.(17)

Most of the regulations that describe health services mandate health service providers not to discriminate or discriminate against health services. The BPJS class classification itself, whose nominal fee is different, in the technical field has a significant impact, especially when compared to the service of no BPJS participants who use public facilities (without insurance costs from BPJS).

However, justice in health services from the perspective of the theory also clearly states that the theory of justice proclaimed by Aristoteles divides justice from two different sides. Justice for Aristotle is a form of ownership of goods. The goods must be owned by every living thing, equally, without exception. (18) The corrective action is aimed at giving birth to new regulations that can later meet the demands of the community for something to be “more” just than before. (19)

The government’s obligation to protect the rights of the public to obtain health services has been fulfilled, but the fulfilment of government obligations to health services that are equitable, fair and without discrimination is not yet fully visible. Frictions that arise in handling BPJS patients due to BPJS compensation in the payment of monthly claims are not in accordance with the INA CBGs Package and there is a delay in disbursement of funds, which can delay (even unpaid) medical and non-medical services for months. These conditions can trigger a decrease in the quality of health services due to rights that have not been fulfilled.(20)

Judging from sociological jurisprudence, according to the theory put forward by Roscoe Pound, the orientation of the view is on the “legal reality” rather than the position and function of law in society. Sociological Jurisprudence reflects a careful blend of written law as the needs of the legal community for the creation of legal certainty (positivism law) and living law.(21)

So that the legislation can run as it should, the legislation that was formed in accordance with what is at the core of the Jurisprudence sociological school that is good law should be in accordance with the law that lives in society.(22) Yet what we have to realize is, the law was made and fully enforced for the benefit of the public interest in society, especially health services. The regulations made did not reflect equality in service.

The Health System in Indonesia, which is now run by BPJS, payment of contributions are divided by class. When referring to the explanation of the article contained in the BPJS Law, it clearly states that the principles of health administration are equal, but in reality, according
to Permenkes-59-2014, it is attached that financing all types of diseases, has a package (maximum) of financing. The bigger the class, the greater the nominal money package allocated by BPJS.

The difference in tariff will inevitably trigger an efficiency on the part of the health provider. Moreover, BPJS has delayed the payment of claims due to (again) underfunded BPJS Health financing. In the community, BPJS patient services for each class are not entirely good. A striking difference is seen in BPJS Class I participants, who can advance to special classes (VIPs) while for Class II and III the maximum will only rise to one level above each.(22)

Compared to developed countries in Finland, Australia and Singapore, most of them attract health funding from taxes. Then there is no known class payment system (I, II, and III). All premiums are the same, depending on the income tax. All citizens are entitled to health services and do not need to worry about the class system. In these countries, all types of services are guaranteed, from promotive to rehabilitative. Even though it is in a state far from the city centre, the community still gets maximum health services, if for example there can also be a supporting examination.(22)

All types of service based on class violate the principle of justice, which prioritizes the principle of equal treatment with one another. In addition, the government also guarantees that it will be responsible for the implementation of health services that are equitable, affordable and safe (prioritizing patient safety). The difference in services can lead to discrimination and the most dangerous is when the act can physically harm the patient.

Conclusion

The class system in the health insurance system in Indonesia can trigger discrimination related to health services enjoyed by the community. The system limits the number of claim payments which causes demands to reduce the quality of health services. These things certainly violate regulations/policies regulated in the laws and regulations of the minister of health, including violating human rights. Reference to the health financing system in other countries can be used as an example to improve the health financing system in Indonesia.

Conflict of Interest- No

Source of Funding-Authors

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Forensic Examination and Restorative Justice for Drug Abusers: An Alternative in Handling Drug Cases in Indonesia

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Abstract

The approach of restorative justice is a new paradigm in responding the crime of drug abuse. So far, the efforts in acting upon drug abuse uses the prohibitionist principle. This approach has the perspective that the drug abuse is illegal and a violation of law, and therefore must be prohibited. The focus of this policy is the efforts of controlling policies in minimizing drug black markets through punitive ways and imprisonment. Restorative justice is the most logical and humane choice for drug abusers who are not involved in the distribution network. They are victims who must be healed and restored, as opposed to being imprisoned. There are many positive effects of drug abuse rehabilitation. The drug abuser will be saved from their problem of addiction of illegal drugs. It is hoped that when they are healed, they can return to the society and socialize with the people as before.

Keywords: drugs; restorative justice; penal; forensic test.

Introduction

Restorative justice is an approach model in the effort to solve criminal cases as a response towards the criminal justice system, which are often thought to not be able to contain the aspirations of the victim and perpetrator.¹ This restorative justice is different from the criminal justice system as it emphasizes the concept of mediation and reconciliation as the mechanism of conflict or dispute resolution in the case of criminal actions. It is something which is nonexistent in the criminal justice system.²

The restorative justice approach is a new paradigm in responding the criminal act of drug abuse. In the perspective of the restorative justice approach, the criminal action is understood as a dispute or conflict which may disturb the relations between an individual and the society. It is not merely understood as a violation of law, where as a consequence, the perpetrator is faced with the state. Thus, justice may only be achieved when the related parties are given a space to participate actively in the process of the drug abuse case to find the best solution which emphasizes healing, reconciliation, and teamwork.

The focus of this policy is the efforts of control policies on minimizing drug black markets through punitive methods or through imprisonment, which are believed to minimize the negative impacts of the drugs. Ironically, this policy will actually cause various violations of the human rights for the drug abuse perpetrator; the violation of the health rights by limiting the access of general medicine and the limitation of health services for prevention, the treatment and handling of HIV, etc.

There are some facts which cannot be ignored when we talk about the violation of the human rights and the arbitrary actions for the addicted people or the drug abusers. This case of policies which emphasize criminalization and which ignore the holistic public health will cause cases which are not easy to be resolved in the future. On 2019, there are 140.423 inmates, 37 295 of them were criminalized for drug abuse. Among them, 285 people died in prison and 89 of them died due to HIV/AIDS.⁴
Table 1. Data of Drug Abuse Perpetrator Classification on 2019

<table>
<thead>
<tr>
<th>No</th>
<th>Month</th>
<th>Perpetrator classification</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Drug dealer</td>
<td>Courrier or distributor</td>
<td>User</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>January</td>
<td>0</td>
<td>101</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>February</td>
<td>0</td>
<td>67</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>March</td>
<td>0</td>
<td>74</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>April</td>
<td>0</td>
<td>70</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>May</td>
<td>0</td>
<td>71</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>June</td>
<td>0</td>
<td>32</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>July</td>
<td>0</td>
<td>74</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>August</td>
<td>0</td>
<td>133</td>
<td>147</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>September</td>
<td>0</td>
<td>66</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>October</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>November</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>December</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>-</td>
<td>688</td>
<td>692</td>
<td></td>
</tr>
</tbody>
</table>

From the case above, it can be concluded that the research problem is, how is the restorative justice as an alternative method in handling the drug abuse cases in Indonesia, and how is the role of forensic examination for drug abusers in the implementation of restorative justice?

**Research Method**

The method of this research was the juridical-normative legal analysis. A legal research was based on the legal concept used. According to Wignyosoebroto, there are five legal concepts, which are:

1. The law is an absolute principle of truth and justice which applies universally;
2. The law is positive norm in the system of national legal constitution;
3. The law is what is decided upon by the *inconcreto* judge, and it is systemized as a judge-made law;
4. The law is patterns of social behavior which is institutionalized and it exists as an empirical social variable;
5. The law is a manifestation of symbolic meanings of social actors and is visible in the interaction between them.

In the second concept of law, there is the normative concept, where in this concept, the law is a norm, and which is identified as a justice which must be manifested (*ius constituendum*)

The norms which is manifested as an explicit order and is positively and clearly formulated (*ius constitum*) to guarantee its certainty. This research combines two approaches, which are the doctrinal/normative method and the non-doctrinal/empirical method, or what is known as the mixed method.
Result and Discussions

The application of the criminal law in Indonesia is as a method to prevent or to handle crime. It does not inherently become a problem if it is able to achieve the main goal which is to create happiness of citizens; a wholesome and cultural living; social welfare; and equality. The use of punitive efforts (sanctions/criminalization) in managing the people through the constitutions are basically part of the stage of policy.9

Meanwhile in the constitutional concept which applies the double-track system, the stelsel sanction simultaneously regulates two things, which are penal sanctions and non-penal sanctions.10 The law regarding drug abuse also has non-penal sanctions, which is an element of the restorative justice for drug abusers, which is rehabilitation until the addiction is healed.

In its development, the world nowadays tends to not see drug abusers as criminal perpetrators anymore. Yet, they are regarded as victims or patients which must be given empathy. The approach of punishment may make the people who need help ironically hide themselves.11 The data shows that on 2019, there are no drug abusers who are classified as users who must be rehabilitated.

Table 2. Drug Case Review Data By BNNP of 2019

<table>
<thead>
<tr>
<th>No.</th>
<th>Criteria of Perpetrator</th>
<th>Number of Evidences</th>
<th>Transferred To Prosecutor</th>
<th>Rehabilitation</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Drug Dealer</td>
<td>5</td>
<td>5</td>
<td>-</td>
<td>All Cases Transferred to Prosecutor</td>
</tr>
<tr>
<td>2.</td>
<td>Courier</td>
<td>10</td>
<td>10</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Distributor</td>
<td>3</td>
<td>3</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>User</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

If analyzed carefully, especially in the medical perspective, there are many parties who think that they are actually victims of a syndicate, or part of the distribution chain of drug trade who have trouble getting over addiction. This may happen even though they actually want to get over drug addiction which binds them. Yet, because their nerves are bound by addictive substances, it is difficult to be done. Users or addicts need a different treatment from other patients in general. There is an urgency for the change of policies in imprisoning drug users and other drug prosecutors. It should be based on the principles of justice, and based on the values of humanity.12

The stipulations on rehabilitation for drug abusers are regulated on Articles 54, 55, 56, 57, 58, and 127 of the Constitution No.35 of 2009 regarding Drugs. One of the principles in criminal law which is parallel with this view is known as the term of restorative justice. It is one of the efforts of legal approach which is used to prevent criminal case resolution. It emphasizes on healing the victim maximally and to return them to the original state.13 This is because in some cases, the sanction of imprisonment is aimed to punish the inmates physically and to give them a deterrent effect. Yet, it does not give a positive benefit in changing the perpetrator and make them deter in repeating their behavior. It cannot guarantee the achievement of healing for the victims.

Restorative Justice is the most logical and humane choice for drug abusers who are not involved in the distribution network. They are victims who must be healed and restored. They should not be imprisoned. The policy in applying the criminalization sanction for drug abusers has so far created new problems which are quite complex, for example, it will affect the condition of the prison.14
Table 3. Inmate Classification in Yogyakarta Drug Prison on 2018

<table>
<thead>
<tr>
<th>No</th>
<th>Inmate Classification</th>
<th>Total</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Drug Dealer</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Distributor</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>User</td>
<td>131</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Producer</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Outside Of The Classification</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>190</td>
<td></td>
</tr>
</tbody>
</table>

In the sociological perspective, the drug users are regarded as acting out from the social norms. Drug abuse may cause death, addictions, diseases, the increase of violence and criminality. It may cause the destruction of the society and may cause a lost generation. In the juridical point of view, drug users who are addicted have on one side violated the law regarding drug usage. This is because there are the legal stipulations on the Constitution on Drugs and Psychotropics which regulates the sanction of imprisonment for perpetrators of drug abusers. If the drug abusers are addicted, they must undergo medical and social rehabilitation. The period of medical treatment and rehabilitation are regarded as the period of undergoing criminal sanction.

The Constitution No. 35 of 2009 regarding Drugs states that the judge which examines the case of drug addicts may undergo two things. First, the judge may give the verdict of ordering the person to undergo medical treatment or healing, if the drug addict is proven to be guilty of drug abuse crime. Second, the judge may give the verdict of ordering the person to undergo medical treatment or healing, if the drug addict is not proven to be guilty of drug abuse crime.

The issuing of SEMA No. 4 of 2010 has given a guide for judges to give a verdict based on restorative justice, which places drug addicts in medical and social rehabilitation institutions. The main consideration for the soul and spirit of the Constitution No. 35/2009 regarding Drugs is the acknowledgement of drug addicts as prisoners. It also protects drug addicts and drug abuse victims by placing them in medical and social institutions.

Table 4. Inmate Classification in Yogyakarta Drug Prison on 2019

<table>
<thead>
<tr>
<th>No</th>
<th>Inmate Classification</th>
<th>Total</th>
<th>Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Drug Dealer</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Distributor</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>User</td>
<td>136</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Producer</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Outside Of The Classification</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>296</td>
<td></td>
</tr>
</tbody>
</table>

Above is the data of prison inmates on 2019. On that year, it is shown that most of the prison inmates from drug cases are users. It shows the alignment of the restorative justice paradigm to place victims of drug abuse in a rehabilitation institution. It has long been voiced and is regulated in the Constitution No. 35 on 2009 regarding Drugs, even though not much has been implemented. Thus, until now, drug abuse victims still have to go through court processes. Drug abuse victims do not need to be captured nor punished. Yet, they need to be given medical treatment and rehabilitation.

The judges’ verdict for the drug abuse victims is right when they are placed in rehabilitation institutions. On the contrary, if they are imprisoned, the drug abuse victims will not obtain rehabilitative treatments. Since there is no treatment, the drug abuse victims will try
to obtain drugs illegally, both through the family or through the drug distribution network in the correctional institutions. This restorative justice is only given to the users. Yet, the drug distributors or drug dealers will still be given penal punishments.

Criminal policies certainly do not only use penal law, yet it also uses non-penal laws. Basically, these two have different ways of working. The effort in preventing crime through the penal method emphasizes “repression” (oppression/elimination/eradication). This form of punishment is given to drug distributors, smuggler, and drug producers. Meanwhile, the non-penal method has the preventive orientation (prevention/deterrence/control) and focuses on curation (healing) which is suitable for users.

According to Pakpahan, the resolution method of restorative justice is more effective than giving sanctions in the form of imprisonment punishments. One of the reasons why restorative justice is more effective in handling the case of drug users is based on the worry regarding the condition of prisons. There are many criminal cases which are given the verdict of imprisonment by the judges, which makes prisons crowded, without the addition of rooms or the betterment of facilities. The rehabilitation of drug users has more positive impacts. For example, the drug users will have a solution for their problem of addiction and it is hoped that when they are healed, they may go back to the society and socialize again as before.

How to differentiate between drug users, distributors, smugglers, and producers? There needs to be a forensic examination to the perpetrators, so as to obtain facts, whether they are drug users, distributors, smugglers, and producers. This is so that the correct action of law may be taken. To detect drug users, forensic examinations may be carried out, including urine tests, hair tests, or blood tests.

The time needed to undergo the urine test form of forensic examination is around one hour. Meanwhile only the lab of BNN and the forensic lab of POLRI can carry out the hair and blood tests. Thus, there is no certain information on the price needed. Another form of forensic examination is by using the Learning Vector Quantization (LVQ). In the research of Sulaiman the forensic examination algorithm uses this method, and it has a high accuracy of 99%. Then in the research of Leleury and Aulele (2016), the Learning Vector Quantization (LVQ) has an accuracy rate of 95,92%. Through this system, it is hoped that the primary identification of drug abusers may be eased, so that it may be treated quickly. It is also hoped to be able to detect and differentiated users, distributors, and producers.

**Conclusion**

Dropping the verdict of imprisonment sanction for drug abusers cannot actually change their behavior and stop them from using drugs. Meanwhile, the deterrent effect which becomes the main aim of penal punishments is not equivalent with the negative effects which are created. Restorative justice becomes the right choice in treating drug abuse in Indonesia, especially for users who need rehabilitation and healing.

The restorative justice approach is a more effective resolution method compared to giving the sanction of imprisonment. One of the reasons why the restorative justice approach is more effective for handling drug users is the worry regarding the supply of prison rooms. There are many criminal cases which are given the verdict of imprisonment by judges which makes the prisons filled. It happens without the addition of rooms nor the betterment of facilities.

The restorative justice method of approach is preferred as it is hoped for by the victims. They want to undergo rehabilitative treatment and return to their previous state. Even though it might not run optimally nor maximally, at least the victims’ rights are guaranteed. The rehabilitation of drug users gives more positive impacts. For example, the drug users may heal from their problem of addiction to dangerous substances. It is also hoped that they may return to the society and socialize again as before after they are healed.

**Conflict of Interest:** No

**Source of Funding:** Authors

**Ethical Clearance:** This article is a literature study, so we don’t need the ethical clearance.

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The Hegemony of The Exxonmobil and Freeport Contract in Investing in Indonesia and Also The Abandonment of The Local People’s Health Rights

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Abstract

The type of contract carried out nowadays, which is the Product Sharing Contract (PSC) and contract of work in the natural resource sector results to product sharing, which is on one hand thought to not yet fulfill the principle of justice for the state. On the other hand, the government still needs funds from foreign investors to carry out economic development. The balancing position between the foreign investment and the domestic investment in the capital market investment in the oil, gas, and the gold-copper mining sector of Freeport in Papua and Exxonmobil in Indonesia shows a foreign domination. It means that there is a natural resource sector investment hegemony by foreign investors. The implication of this condition is the lightening burden of the state budget, the increase of workforce absorbment and the transfer of technology. It also causes opportunity cost. There is the environmental destruction, including the aspect of healthcare for the local people in the mining location as a compensation. For that, the government needs to review the contract regulation for these two natural resources and revise what is necessary.

Keywords: hegemony, investment, contract of work, production, product sharing contract, health.

Introduction

Sources of oil and gas are potential reserves of energy wealth since Indonesia was still invaded by the Dutches until the government in the Reformation era. There have been explorations and exploitations by foreign contractors which are divided into blocks of oil and gas management, as stated by Arifin1. According Mubyarto2 Until now, this cooperation contract is still maintained, even though it often causes problems. Now, the government still makes sure that the management of natural resources which require high capital, high technology, high skill, and of high risk, which are almost impossible to be done independently by domestic contractors, may still be done through a cooperation with foreign contractors, as stated by Suherman.3

According to Dimyati4, the sociological impact which appears in the period of contract renegotiation is that the politicians, groups of society, and the people who live around the mine areas were preoccupied by the pro-cons of the parties who supported the extension and those who did not with their own argumentations. This is very reasonable, remembering that on the side of the contractor, with the contract extention, the capital gain of their investment will continue to be received, as said by Adolf5. Meanwhile, on the side of the local people, especially those who receive direct impacts from the mining project, will feel that the compensation received is not equal with the social, environmental, and health sacrifices they had to make, as opined by Asshiddiqie6. Thus, the welfare of the local people is relatively stagnant. There is no improvement. Worse, there are environmental deterioritations which are difficult to fix.

In the economic point of view, the natural resource management cooperation contract with the foreign companies is the hope of the government. The government wishes this investment to be continued and to even be increased, considering that the foreign investment may
lighten the burden of the state budget for infrastructure development, said Ahmad 7. It may also increase the work opportunity for the local workforce. ExxonMobil and Freeport are the largest foreign contractors in the natural resource management sector compared to other companies which invest in Indonesia. The two contribute quite a large portion of capital compared to that contributed by the domestic companies in the same sector.

The data from the Capital Investment Coordinating Board (Badan Koordinasi Penanaman Modal/BKPM) shows that on 2017, the total investment was Rp 692,8 trilyon. This number is divided into that from foreign investment, which was Rp 430,5 trilyon, and domestic investment which was Rp 262,3 trilyon. Thus, the ratio between foreign investment and domestic investment was 62,1:37,9. The sectors of water, oil, and gas (including that managed by ExxonMobil), and electricity contribute 11,8%, meanwhile mining (including that managed by Freeport) contribute 11,4% out of the total investment, as stated by Mubyarto 8. From the 24 existing investment sectors, oil and gas, electricity, and water stands in the first rank of investment income, whereas mining stands in the second rank. This condition indicates the foreign domination in the investment in Indonesia.

**Method**

This research is a juridical-normative study. It uses a literature review approach. As said by Rusli 9, this research uses a qualitative analysis in analyzing the present problems.

**A. The Extention of Natural Resource Contract**

The Constitution No. 25 of 2007 regarding Investment is one of the government’s regulation in the investment sector, which aims to accelerate the development of the national economy. If it succeeds, it will create success for the state of Indonesia, which will then bring its people towards a higher and more widespread level of welfare. As good business actors, both foreign and domestic investors are encouraged by the government to realize that aim.

In the natural resource sector, the phenomena which happened was the fight for the power to manage it. This means that there was a competition between foreign and domestic business actors in investing in Indonesia. The sector of oil and gas and the sector of mining were two of the many natural resources which were fought for by the investors. Even so, considering that to run the business of these two sectors need much capital, sophisticated technology, special skills, and contains high hazard, foreign companies still dominate this sector, as stated by Wahab 10. ExxonMobil and Freeport are the two foreign contractors in the oil and gas and mining (gold-copper) sector, which have dominated those natural resources from a long time ago up to now, even though their presence in Indonesia still causes much problems in the implementation and in the substance of the contract. According to Redi 11, to minimize the unwanted things and so that investment still goes on safely and so that it reaches the aim, the government regulates it through adequate regulations, which are the Constitution No. 22 of 2001 on Oil and Gas and the Constitution No. 4 of 2009 regarding Mineral and Coal Mining.

Even though all oil and gas management contracts are based on the Oil and Gas Law, and that the mining of gold and copper is already based on the Mining Law, there are still problems here and there which needs extra attention from the government. Problems appear when the contractors (both foreign and local) face an expiring contract, where they will undergo a renegotiation for contract extention. In this case, even though the renegotiation has been agreed upon by the contractors and the government, the substance and the implementation of the contract are not always in line with the hopes of the government regarding investment policies, which is the balance between foreign and domestic investors, as said by Hodd in Newcomb 12.

**B. Legal Protection of the Indonesian Natural Resource Management**

The management of natural resources in Indonesia is based on the legal protection in which its source is the 1945 Constitution. On Article 33 paragraph (2), it is stated that the important branches of production for the state and that which has the power over many people’s interests are under the power of the government. Meanwhile, paragraph (3) states that the earth, water, and natural resources in it are under the power of the state, and must be used maximally for the welfare of the people. Meanwhile, the management of every kind of natural resource, including oil, gas, and gold-copper mining is regulated on some of its own Constitutions, which are derived from the 1945 Constitution.

The Indonesian oil and gas management was first based on the Constitution No. 44 of 1960 article 2 which
states that the oil and gas mining materials which are in Indonesia’s legal mining territory are part of the national wealth which is under the power of the state. Then, in its development, it is now regulated on the Constitution No. 22 of 2001 regarding Oil and Gas. Meanwhile, for the gold-copper mining, it is regulated on the Constitution No. 4 of 2007 regarding Mineral and Gas, as stated by Redi11.

C. ExxonMobil and Freeport in the capital market

In the beginning, the oil and gas contract in Indonesia was a contract of work. Then, since 1971, it implements the product sharing contract, as stated by Partowidagdo.13 Then, after going through some changes in the contract model, the Constitution No. 22 of 2001 regarding Oil and Gas was issued as the legislation which regulates the oil and gas management. Since the issuing of that constitution, the management of oil and gas by foreign contractors are carried out through a contract of work with the product sharing contract (PSC) model.

ExxonMobil which has operated since 1899 is one of the tens of oil and gas contractors which invested their capital in Indonesia through the PSC system, said Mubyarto2. The latest data (2019) showed that there was the domination of foreign contractors in the management of oil and gas, with foreign contractors managing 70% of the sources of those sectors in Indonesia. With ExxonMobil’s contract extention, its contributions towards the conductivity of capital investment in Indonesia is highly significant, even though there are still problems in its implementation and product-sharing, as stated by Partowidagdo13.

ExxonMobil’s contract extention gives a huge contribution towards the foreign investment in Indonesia, even though it indicates that Indonesia’s sovereignty in managing oil and gas is not possible yet. Apart from that, it is highly ironic that the oil and gas, which are natural sources which support the lives of many people are not treated as the state’s strategic commodity anymore, as its management is regulated according to the wishes of the foreign contractors. The impacts of this condition are felt by people of Cepu, Bojanegara, who live around the oil and gas blocks managed by ExxonMobil. They feel that they are not yet living a prosper life. Their area experiences environmental destruction and the people’s health rights are disturbed. These things should be covered by the ExxonMobil company for the people surrounding the mines.

Freeport, a gold-copper mine foreign contractor, has carried out a contract extention the third time after fifty years of managing the oil and copper mines in Timika, Papua. Freeport was the first company to enter Indonesia some time after the G30S PKI phenomenon which forced President Soekarno to step down from presidency. The legal basis of the first-generation work contract at that time was the Constitution No 1 of 1967 regarding the Foreign Capital Investment with the period of 30 years since it first operated on 1973 until 1999, said Partowidagdo13. On 1991, the second-generation contract of work was issued for the period of thirty years (1991-2021). Then, after the contract period ran out, the contract status was changed into the Special Mining Business Permit (IUPK) based on the Governmental Decree No. 1/2017 with the period of ten years, and may be extended for another ten. Freeport contributes 5% of the total investment in Indonesia.

The positive implication of the contract agreement, which has now changed into the Special Mining Business Permit, was the reemployment of around three thousand people, the majority of them from Biak, Jayapura, Sorong, Serui, which was formerly fired. Meanwhile, the negative implications include environmental deterioration, as stated by Mubyarto8. The Amungme tribe has also felt being out of place in their own land, as their positions were pushed by the presence of foreign mines. The health factor of the Amungme people has not improved either, as there is still a high rate of infant and maternal mortality. They are prone to diseases, and there is still a low rate of the Amungme people’s survival said Syeirazi14.

The issuing of the constitution on Capital Market has brushed aside the beliefs that the foreign companies’ presence in investing in Indonesia imply that: (1) there is the decrease of the savings rate or domestic investment by creating an unhealthy competition from exclusive contracts between the multinational companies and the Indonesian government; (2) reinvestment is not reached from the income obtained.

D. Irony of the Natural Resource Contracts in Indonesia

The constitutional regulations in Indonesia are created in the form of constitutions which reflect on the 1945 Constitution. The main part of the constitutional regulations, which are the substance of this research are
the constitution on mineral and oil natural resources, the constitution on minerals and coal, and the constitution on investment, according Syirazi. In the economic perspective, the writer is pessimistic that with the regulations on natural resource management contact extension which still do not support the interests of the people, the results of the oil and gas in the blocks and in the gold-copper mines in Papua will succeed in increasing the welfare of the local people. Based on the existing data, the extent of the natural resource contact extension is identical with shifting 70% of its outcome to the foreigners, which means that only 30% of it may be enjoyed by the surrounding people, said Aviliiani. Worse, there is still the environmental factor, and the abandonment of the local people’s health rights which are disturbed by the mining activities, and which do not receive any attention from Exxonmobil and Freeport, said Newcomb.

The data from the Capital Investment Coordinating Board shows that during the period of 2017, the amount invested by foreign investors were Rp 430.5 trilyon, and that from domestic investors were Rp 262.3 trilyon. Thus, there is a ratio of 62.1: 37.9. This shows the domination of foreign investors in investing in Indonesia, said Partowidagdo. The water, oil and gas, and electricity sectors contribute 11.8% of the total investment, whereas the mining sector contributes 11.4%. From the total of 24 investment sectors, the sectors of oil and gas, electricity, and water stands in the first rank, with mining following on the second rank of investment income. This means that ExxonMobil and Freeport, which are the largest American companies in these sectors dominate in the largest investment sectors in Indonesia, according to Sycirazi. This condition is suspected to be an impact of the consequent implementation of the Constitution on Capital Market, in which one of its articles states that it does not differentiate foreign investors based on their country of origin. As an impact, if there is no control from the government, there will be a continual hegemony of foreign investors towards the domestic ones, which means that there will be an unhealthy imbalance between the two types of capital investment.

Conflict of Interest: No

Source of Funding: Author

Ethical Clearance: This article is a literature study, so we don’t need the ethical clearance.

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Stage III-B Cervical-cancer of Young Age in Medical, Bioethics and Clinical Ethics Perspectives

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Abstract

Background: The incidence of cervical-cancer in Indonesia is mostly obtained at the age of 40-50 years. But now many are found at the age of 25-40 years. Method: Case-report. Findings: Female, 25 years old with a diagnosis of cervical-cancer stage 3B planned for chemotherapy. Patients complain of vaginal bleeding. Married 2 times, at the age of 16 years and 19 years with infertility. The central dilemma: a young woman with advanced stage cervical-cancer was planned for chemotherapy as a gold standard, in terms of patient-autonomy and family refusal. Informed consent was required in the delivery of bad-news and human approaches based on Kubler-Ross’s theory (denial, anger, bargaining, depression, acceptance) with the concept of SPIKES (Settings, Listening-Skills, Patient’s Perception, Invitation to share Information, Knowledge transmission “bad-news”, Explore Emotions and Empathize, Summarize and Strategize). Both medical indication and quality of life were appropriated but in terms of patient preference and contextual feature were refused by patient and families. Resolving this case was not enough with medical-aspects, it also required a bioethical and clinical ethics approach. Conclusion: Medical, bioethical and clinical ethics approaches are methods of resolving a case with an ethical dilemma.

Keywords: cervical-cancer, perspective of medical, bioethics and clinical-ethics

Introduction

According to WHO (2012), around 8.2 million deaths were caused by cancer. Ministry of Health reported the number of cancer sufferers in Indonesia reaches 6%. Based on data from the Ministry of Health, the Indonesian Cancer Foundation, and the Indonesian Association of Pathologists, 64.4% of cancer suffered by women.1,2

The high mortality rate because most of cervical-cancer sufferers were aware of the disease after being in an advanced stage, because in the early stages the patient did not feel any complaints or symptoms. If it was at an advanced stage, cervical-cancer would cause more physical complications and death. Cervical-cancer is a type of cancer that is caused 99.7% by oncogenic human-papilloma-virus, which attacks the cervix (Indonesian Journal of Cancer, 2009). In Indonesia, only 5% patients did the screening for cervical-cancer, while 76.6% of patients had entered an advanced stage (III-B and above) when detected because cervical-cancer is usually asymptomatic at its initial stage.2,3

Method

The method of this study was case-report.

Findings

A 25-year-old woman escorted to Wahidin Sudirohusodo Hospital by her sister for chemotherapy. Patients diagnosed with stage III-B cervical-cancer with complaints of vaginal bleeding for 3 months that had not
stopped, she also had a history of post-coital bleeding 6 months ago. Two times marriage history at the age of 16 years with a 3-year old marriage, divorced and has no children, the second husband last 1.5 years, has no children as well. No history of contraception. She worked as a cafe/waitress assistant, her husband is still alive and works as a driver.

The physical examination: vital-signs=normal, good general condition, compos mentis, BP=100/60 mmHg, pulse=82x/minute, RR=18x/minute, T=36.5°C, height=149 cm, weight=51 kg, BMI=22.97 kg/m². Gynecological examination: high palpable uterine fundus, tumor mass and pain were absent, blood flux was present. Speculum examination found no vulva abnormalities, the vagina looked bumpy at 1/3 proximal, the portion was lumpy, fragile, easily bleed, blood flux was present. The results of examination in the vagina, obtained 1/3 proximal palpable mass with fragile bumps, easily bleed mass on the entire surface of the portio and parametrium.

Ultrasound examination: mass in the lower segment of the uterus measuring 4x5 cm, uterine size slightly enlarged 7x6x5 cm in size, both adnexal normal, no free fluid appeared in the Douglasii cavity, the kidney appeared hydronephrosis bilaterally. The diagnosis of this patient was Stage III-B Cervical-cancer Pro ChemoTherapy.

**Discussion**

**Medical-Analysis**

The quality of palliative-care focuses on client-choice, collaboration, interdisciplinary and holistic approach and orientation to the client and family. During the recovery-period, support for the client and family is a focus in curative and non-curative services, this is known as supportive care. Supportive care services for the physical and psychosocial can be provided in the form of education, research and promotion of clinical services and improving the quality of life of clients with cancer.4,5

Based on 30 analyzed literature, concluded that changes occur in an end-stage cancer causes changes in quality of life because quality of life consists of four dimensions namely physical, psychological, social relationships and environmental dimensions that are not only dealt with curatively but need a more personal approach so that it can be concluded that palliative-care plays a role in achieving maximum quality of life in stage IV cancer thereby reducing pain or preparing for death. Good palliative-care can change the life quality of cancer-sufferers for the better. However, palliative-care are still rarely performed in hospitals in Indonesia, because focuses are still on curative, while physical, social and spiritual changes cannot be intervened entirely with curative. In order to make the life quality of cancer patients remain high, there are several things that need to be done, among them is by implementing a comprehensive and integrated palliative-care from the palliative team.6

Kubler-Ross state that there are five stages of emotional reaction that are associated with chronic-diseases: denial, anger, bargaining, depression, and acceptance.7

a) Denial

Denial is a defense system that makes a person try to avoid the effects of illness.

b) Anger

The patient tries to question “Why do I have to suffer from a chronic illness?” Analysis: the patient is in this stage because the patient feels that she still has a lot of hope that she wants to do in the future, for instance having children, enthusiasm in finding fortune, but this disease makes her hopes disappear.

c) Bargaining

The cancer-sufferer divert anger better with different strategies.

d) Depression

Depression as a lack of control is a realization of the worsening of symptom as a condition of a disease that does not improve.

The cancer-sufferer will feel fed up, tightness, fatigue, difficulty eating, difficulty controlling herself, difficult to focus attention, avoid pain and feeling uncomfortable.

e) Self-acceptance

The cancer sufferer is no longer angry and has familiarized herself with the idea of death which makes her depressed and also faces unpleasant thoughts.
This patient was in the anger stage, it was evident in every time we provide education related to the disease, the patient had difficulty concentrating and unstable emotions, there was an acceptance stage to start chemotherapy due to coercive factors from parents.

KODEKI (Indonesian Medical Code of Ethics) states that “Any doctor’s actions or advice that might weaken psychic or physical endurance, must obtain the patient’s/ family’s approval and are only given for the benefit and good of the patient”. To reduce drastic emotional changes of patients, the delivery of information about their illnesses is carried out with the “SPIKES”:

1. Setting, Listening-Skills

Before delivering bad-news to patients, it is necessary to prepare ensuring the smooth delivery of information to patients before delivering bad-news, you should prepare the ability to ‘hear’, include: silence, repetition, availability.

2. Patient’s Perception

Before delivering bad-news, the doctor should know the patient’s perceptions of: her own medical condition and her expectations of the results of the medication she was taking, ask the patient’s estimate of the results of the medication

3. Invitation to share Information

Ask whether the patient wants to know the developments regarding her situation or not. If the patient claims not to be ready, consider delivering at a more appropriate time and ask the patient to prepare in advance. If the patient states that she wants to know the progress of the situation, ask the extent to which she wants to know.

4. Knowledge transmission “Delivering bad-news”

Before delivering bad-news, do a ‘warning shot’ as an opening to tell the patient that there is ‘bad-news’ to be delivered to the patient so that the patient is not surprised.

5. Explore Emotions and Empathize

Always observe the patient’s expression and emotion as well as what underlies changes in her emotion, examine the patient’s emotional state.

6. Summarize and Strategize

At the end of the talk, review the entire conversation again: conclude the ‘bad-news’ that was delivered in stages (piecemeal). Conclude the response given by the patient during the bad-news delivered, show that the doctor listens and understands what the patient is delivering, gives the patient the opportunity to ask questions, gives feedback, and discusses plans to follow up on the bad-news that has been conveyed to the patient.

Bioethics Analysis

As stated in the Geneva Declaration the doctor stated: “Patient health will always be my first consideration” and the International Medical Ethics-code states: “Doctors must give their patients full loyalty and all the knowledge they have”. The doctor must tell the patient the consequences of the decision taken. Patient must understand clearly what the goals of a test or treatment are, what results will be obtained, and what the impact would happen if they delay the decision.

The rules of bioethics are an absolute law for doctors. The Indonesian Medical Council, by adopting the principles of western medical ethics, stipulates that the practice of Indonesian medicine refers to 4 basic moral principles which are often also called the basic principles of medical ethics or bioethics, namely:

1. Beneficence

It’s a principle that a doctor is doing good, respecting human dignity, the doctor has to make every effort so that the patient remains in good health. The principle of beneficence emphasizes the role of the doctor to provide convenience and pleasure for patients in taking positive steps to maximize the good results rather than the bad things. The principles contained in this rule are: prioritizing altruism, guaranteeing the basic values of human dignity, looking at a patient or family is not an action that only benefits the doctor, trying to have more good or benefit compared to a bad one, paternalism is responsible/compassion, ensuring the good and minimal life of humans, maximizing the rights of patients as a whole, implementing the Golden Rule Principle, giving an efficacy but inexpensive prescription, developing the profession continuously, and minimizing bad consequences.

Analysis: the doctor has done the best for the
patient in the treatment effort by doing chemotherapy in accordance with the procedures of medical-treatment in hospital which all costs are borne by the Government (BPJS).

2. Non-Malfeasance

Non-maleficence is a principle in which a doctor does not perform actions that aggravate the patient and choose the treatment with the most minimal risk for the patient who is treated by him. The ancient statement, first, do no harm, still applies and must be followed. Non-maleficence has the following characteristics: helping emergency patients, treating injured patients, not killing patients, not looking at patients as objects, not insulting/abusing/utilizing patients, protecting patients from attacks, patient benefits outweigh the doctor’s losses, no endangering patients because of negligence, avoiding misrepresentation, giving life spirit, not committing white collar crime.

The principle applied to this patient is that when chemotherapy is performed, the patient is given premedication drugs so that in the process of chemotherapy the side effects are minimized.

3. Autonomy

Every individual must be treated as a human who has the right to self-determination. The Autonomy rule has the following principles: respect the right to self-determination, honestly respect privacy, maintain patient secrets, respect patient rationality, carry out informed consent, allow adult and competent patients to make their own decisions, not intervene or impede patient-autonomy, prevent others from interfering with patients in making decisions-including the patient’s own family, patiently waiting for decisions to be taken by patients in non-emergency cases, not lying to patients even for the patient’s benefit, and maintaining relationships or contracts.

Analysis: After receiving counseling, information and education about the disease related to complications and prognosis, the patient chose to undergo chemotherapy. Autonomy requires that patients must first receive and understand accurate information about their condition, the type of proposed medical-treatment, the risks, and also the benefits of the medical action.

4. Justice

Justice has the following characteristics: applying everything universally, taking the last portion of the process of sharing that he has done, providing equal opportunity to individuals in the same position, respecting the health rights of patients, respecting the legal rights of patients, respecting the rights of others, maintaining the rights of others vulnerable groups, do not differentiate services to patients on the basis of SARA, social status, etc., do not abuse, make contributions that are relatively equal to the needs of patients, request patient participation in accordance with their abilities, return rights to their owners at the right time and competent, not giving a heavy burden unevenly without valid or appropriate reasons, respecting the rights of the population with the same vulnerable to disease or health problems, and wise in macroallocation.

Analysis: doctors provide the same treatment of chemotherapy services to all patients who need these services for the benefit of patients without looking at the economic, social-status, elements of SARA and so forth.

Clinical-Ethics Analysis

Jonsen, Siegler and Winslade (2002) develop ethical-theories that use 4 essential topics in clinical services, namely:\textsuperscript{13,14}

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<td>Nature of disease</td>
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(Source: Mappaware NA. Bioethics, Medical Law, and Human Rights. Umitoха: Makassar; 2010)

Figure 1. The essential topics in clinical-services
1. Medical-Indication

All relevant diagnostic and therapeutic procedures are included to evaluate the patient’s condition and treat it. Ethical questions on this topic are similar to all information that should be conveyed to patients in the doctrine of informed-consent.

After answering all the above medical indication questions using the principle of beneficence and non-malificence, it is determined the choice of advanced chemotherapy to prevent further complications of metastasis to the surrounding organs.

2. Patient-Preferences

The ethical questions include questions about the patient’s competence, volunteer nature, attitudes and decisions, understanding of information, who makes the decision if the patient is incompetent, the values and beliefs held by the patient, and others.

In this case it is more the autonomy principle where the patient is mentally capable and legally competent in realizing and understanding her current clinical condition so that she agrees to do further chemotherapy. Previously the patient had understood the advantages and disadvantages of this chemotherapy procedure through informed-consent.

3. Quality of Life

This is the actualization of one of the goals of medicine, which is to improve, maintain or increase the quality of human life. What, who and how to assess quality of life are ethical questions around prognosis.

According to WHOQOL (1996), that there are some things that need to be considered when assessing life quality, where life-quality is closely related to the domain being assessed, namely: 1) physical-health, 2) psychological, 3) degree of independence, 4) social-relations, 5) environment, 6) spirituality/religion/ individual beliefs. To find out how one’s quality of life can be measured by considering the physical, psychological, social and disease conditions.15

According to Ferrell et al. in Zeng (2010), there are 4 domains in measuring life quality of cancer patients: 1) physical-control or relief of symptoms and maintenance of function and independence, 2) psychological (efforts to maintain life control over threatening diseases marked with emotional disturbances, changes in life priorities, fear and positive life changes), 3) social (efforts to deal with the impact of cancer on individuals, their roles and relationships), 4) spiritual (ability to maintain the hopes and meaning of a cancer experience which marked by uncertainty).16

4. Contextual-Features

The principle in Contextual Features is Loyalty and Fairness. Here ethics questions are discussed around non-medical-aspects that influence decisions, such as family, economic, religious, cultural, confidentiality, resource allocation and legal factors.

The patient’s own motivation to run chemotherapy is still lacking. There are no obstacles in making good decisions from the family, all support her in undergoing treatment. There are no religious, cultural or belief factors that conflict with decision-making.

Conclusion

Medical-treatment has been carried out in accordance with medical-aspects, bioethics and clinical-ethics.

Funding-Authors

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Ethical Clearance- Yes

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Malaria Prevention and Eradication Program Towards Malaria-Elimination in West-Seram

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Abstract

Malaria is a health problem in the world spread in 95 countries. The research objective describes the evaluation of malaria-prevention and eradication programs including inputs, processes, and outputs using qualitative approach. Data were collected by in-depth interview. Research informants were 1 key-informants and 4 supporting-informants. Malaria-elimination program in West-seram District based on the guidelines of the Ministry of Health of the Republic of Indonesia and operationally outlined in the form of a technical guide. Case surveillance has not been carried out. Malaria vector control was done by eradicating mosquito nests and using mosquito nets containing insecticides. Cross-program and cross-program cooperation had been going well, but cross-sectoral collaboration, still needs to be improved. The funding was largely supported by donor agencies namely the Global Fund for AIDs, TBC, and Malaria (GF-ATM) while Government of West-Seram allocates microscopic slide examination costs. It is necessary to increase health personnel, facilities and infrastructure, discovery, prevention, and case management.

Keywords: malaria, elimination, evaluation, prevention, eradication

Introduction

Global Malaria Program (GMP) states that malaria is a disease that must be continuously monitored, monitored and evaluated and the appropriate formulation of policies and strategies is needed. The Global Malaria Program aims to reduce the burden of global disease by 40% by 2020, and by 90% by 2030.(1)

The Indonesia Government establishes efforts to eliminate malaria in Indonesia through Health Minister Decree No.293/MENKES/SKI/IV/2009 regarding malaria-elimination, so malaria is a complex problem so malaria-elimination must be carried out in an integrated manner by all related components and become part of national development. So that the realization of healthy people who are free from malaria gradually until 2030.(2)

Malaria cases in Maluku Province in 2015 was 1,332 positive cases, with a morbidity-rate/ Annual Parasite Incidence (API) of 3.2 per 1000 population. MDGs target for API 2015 is <1%. 2014 as many as 1086 positive cases with API of 4.5 per 1000 population. This means that for 2015 there was a decrease in the number of malaria-cases, although it did not show a sharp decline. Health Profile of West-seram Regency in 2018, positive malaria-cases in West-seram in 2015 were around 1087 cases. This number decreased when compared with positive malaria-cases in 2014, which were 1915 cases. The West-seram administratively has 11 Subdistricts, of which 7 of the 11 districts are malaria-
endemic areas, the sub-district with the highest malaria-endemic is Huamual. Positive malaria-cases were based on a microscopic examination of 1087 cases. The sub-districts with the highest endemicity were found in the village of Uweng Beach in 2015 (546 cases). In 2016, the highest malaria-cases occurred in Talaga Kambelu, which was 120 cases. In 2017, Tanah Goyang sub-village was in the first place of malaria-cases with 256 cases and in January to July 2018 the highest number of malaria-cases occurred in Talaga Kambelu (15 cases). (6)

Based on API of West-Seram, the highest malaria-incidence in the last five years occurred in 2015 (55.60 per million). Whereas for the Annual Malaria Incidence (AMI) indicator in West-seram, the highest malaria-incidence rate based on clinical-symptoms occurred in 2015-2018 occurred in Talaga Kambelu with the highest occurrence in 2015 (85.88 per million)(6)

The malaria-elimination program in the regency is carried out thoroughly and integrated by government together with partners and the community. Malaria-elimination program was carried out in 18 health centers in sub-districts. The Government through the primary health care has done various things, starting from finding malaria sufferers, establishing malaria diagnoses through microscopic examination, treatment using Artemisinin Combination Therapy (ACT), treating malaria in pregnancy, provision of facilities (microscopy, Rapid Diagnosis test/RDT) for laboratory materials and medicines, distribution of mosquito nets, improvement of human-resources quality and eradication of mosquito breeding-sites.(6)

This study aims to describe malaria-prevention and eradication programs based on input, process and output.

**Method**

This type of research was qualitative study, involved 1 key-informant (the program manager at the West-Seram Health Office) and supporting-informants namely: the head of the West-Seram Health Office, 1 surveillance officer, and 2 community members living in West-Seram. The study was conducted in September to October 2018. Data validity was done by triangulating data sources. Stages of data analysis: 1) writing interview transcripts, 2) reading and reducing data, 3) providing data codes based on the meaning of data 4) drawing conclusions.

**Findings**

Respondents were 2 people consisting of supporting informants and key-informants. It was found that all informants had the same answer to the main questions about the malaria-elimination program. Another thing was revealed from interviews:

“The malaria-elimination program in West-Seram began in 2006 in collaboration with the central government through GF and began in 2007-2008. The background to the malaria-elimination program is that malaria-cases are very high in West-seram”(BT,PA).

Next question;

“For an organization in the malaria-elimination program in West-seram in the head directly by the Head of the Health Office then under the head of the malaria-elimination program office is headed by the head of the malaria-elimination program field. In addition, the elimination process also involves various health workers such as the program manager in the DHO, MCH and immunization program manager. For facilities and infrastructure for malaria-elimination in West-Seram, it is financed by two sources, GF and the Government of West-Seram” (BT,PA)

**Facilities and infrastructure**

“Every primary health care in West-seram is required to have a laboratory and laboratory personnel. However, most primary health care in the district do not have laboratories and laboratory personnel, so most malaria examinations in primary health care using RDT. Of the 17 health centers in West-seram that have trained/trained laboratories: Kairatu, West Kairatu, Waimital, Piru, Waisala, Taniwel” (BT)

“Other health centers do not have laboratory personnel and malaria testing still uses RDT system”.

“There are no specific policies issued by the Head of the Local Health Office, in the form of malaria-prevention programs in West-seram District, both in the form of standard operating procedures (SOP) and implementation guidelines (operational guidelines). “However, in the planning and implementation of malaria-prevention programs, the health department compiles technical guidelines (Technical Guidelines) which contain, among others, the objectives of the program, program indicators, types of activities and activities funded for a period of one year. Technical
guidelines compiled by the health office become a reference for the implementation of malaria-prevention programs at the level of health centers and health offices. The reference used to compile the Technical Guidelines is the malaria-prevention program guidelines from the center, in this case, the Ministry of Health of the Republic of Indonesia” (BT).

Process: Discovery and management of patients

“The mechanism of finding malaria is seen from the health center data. There are two activities carried out by the government in the malaria-elimination program, namely active activities and passive activities in terms of activities inside and outside the building. Passive activities in the form of health services only accept or wait for malaria sufferers to come for a check-up while active activities or outdoor activities are Mass Blood Survey (MBS) activities with a record that all people in West-Seram Regency must be examined and MFS activities that are only in the form of searching for the sick. The sick person in question is only people with malaria-symptoms who are examined not everyone. How to find people with malaria-symptoms is done by asking the community about people who have malaria-symptoms”.

In the next question;

“In the malaria-elimination process, the most influential obstacle is funding. Because of this lack of funding there are still many planned programs that have not yet been implemented. In addition, the geographical location of the health center is also very influential, for example, the distance and the road taken is not good. In addition, there are also some primary health care that are located if they have to use a motorized boat to cross the road so that the access of health workers to the primary health care is a bit difficult and the mobility of sufferers from their homes is still relatively difficult to reach a more adequate health service center”.

“In addition, obstacles from the patient’s side are not compliant or routine taking medication and do not follow re-examination. Every patient who has been taking malaria medicine for three days must return to health services to do a re-examination. The lack of awareness among the people that causes malaria in West-Seram Regency is still fairly high” (BT).

Malaria-elimination program in West-Seram are: 1) Discovery and management of patients (discovery, treatment), 2) Prevention and control of risk factors (distribution of insecticide-treated bed nets, health promotion), 3) Surveillance epidemiology and epidemic prevention (SKD-KLB reporting, prevention of outbreaks, malaria information-systems), 4) Improvement of Communication, Education and information (cross-sectoral coordination and cooperation in malaria-elimination), 5) Increasing human-resources (training for health workers and microscopic personnel).

Prevention and control of risk factors

“The prevention and control of risk factors are carried out by spraying the walls of houses or IRS, but IRS activities are also still ineffective due to funding constraints. In addition, there are activities in the distribution of mosquito nets in each health center and other activities, namely the distribution of abate powder and IRS”. (BT)

“West-Seram District last experienced an outbreak of malaria in 2010. During the last 8 years (2011-2018), although malaria-cases were fairly high, it did not reach the KLB. The information system in Seram Barat Barat has four health centers that have been trained, namely, Tomalehu, Kairatu, Kairatu Barat and Piru. The most perceived obstacle in the information system is that it is difficult to signal, especially for primary health care which is located in remote areas”. (BT,PA)

Information Education Communication Improvement

“To increase IEC, it is carried out under the supervision of GF. Supervision is carried out in the form of direct supervision of officers from the health center level starting from reporting on laboratory supervision and work processes carried out at the health center” (BT,PA).

Increase in human-resources

“Human-resources have been trained, especially for microscopic personnel. In minimizing the obstacles to the malaria-elimination program, the malaria-elimination program section of the West-Seram Health Office is working with the planning department to coordinate every activity proposed by the program manager. Malaria-cases in West-seram have decreased from 2016 8.00 / mil and in 2017 to 4.92 / mil”. (BT,PA)

It was found that malaria-elimination in West-seram chose 4 stages:
a. Eliminating criteria, not all primary health care have been trained with microscopic staff

b. Before eliminating the criteria, all officers have been trained and 100% have taken medicine

c. Eliminating criteria, all primary health care still have malaria but with a very small percentage.

d. Maintenance is no case (0%) (PA, 05 October 2018)

Output

“The hope of the implementation of malaria-elimination in the West-seram District Health Office is to reduce malaria positive cases and the target of malaria-elimination in West-seram in 2021 can be achieved. Suggestions and funding, as well as community participation in supporting malaria-elimination programs, are highly expected by the government because to date community awareness and community participation is still lacking” (BT,PA).

Discussion

The goal of malaria-elimination in West-seram will be achieved in 2021 so that in that year it is expected that a healthy living community is free from malaria transmission.(2) There is no specific organization for the malaria-elimination program in West-Seram, the organization still uses the structure of the West-Seram Health Service. The malaria-elimination program in West-Seram began in 2006 in collaboration with the central government through the GF. Guidelines from the Ministry of Health (Technical Guidelines) as operational guidelines that contain goals, targets, indicators, and budgets sourced from the Government of West-Seram for malaria control.

Process

The implementation of the discovery of malaria sufferers in West-Seram was carried out passively and actively. Case finding is passively carried out through services at primary health care while waiting for patients who come for treatment. Patients who come to the clinic with clinical-symptoms of malaria, blood is drawn and examined microscopically. Whereas patients with clinical-symptoms of malaria who come to the primary health care have their blood drawn and blood tested using RDT.(7)

Patient discovery is actively carried out in areas with cases that are still high by means of MBS. This is in accordance with the guidelines for malaria management, that the method of finding patients can be done through these activities(6). The patient discovery activity in West-Seram is the same as the activity carried out in the Principe Islands, Taiwan, with PCD and MBS to support malaria-elimination efforts in Taiwan. Malaria sufferer discovery activities in Purworejo, besides PCD, ACD activities are carried out not only by SBM but through active search activities in the community by the Village Malaria (JMD).(9)

Proof of malaria based on PfMSP-1 allele K, MAD20 and RO33 polymorphisms found in closed communities living in malaria-endemic areas. Early-detection and screening are very helpful in eliminating malaria.(10) Laboratory tests to determine malaria diagnosis are carried out microscopically and using RDT. Microscopic examinations are carried out if the patient comes to primary health care, whereas RDT are done if the patient with clinical-symptoms comes to the nurse. Laboratory tests carried out in West-Seram are in accordance with the standards set by the Ministry of Health, even though the gold standard is microscopic examination. The guideline in conducting standard diagnosis is that every case of suspected malaria must be examined by a blood supply using a microscope or RDT.(2)

The role of the local government in the malaria-elimination program includes mobilizing potential resources including funding support. The results showed that the West-seram Government Fund for the implementation of malaria control programs was minimal and the budget provided for microscopic slide examinations by primary health care in endemic areas. The source of funds for the discovery and treatment of sufferers and vector control by the use of insecticide-treated bed nets comes from donor agencies, the GF.(11)

Output

West-seram is an area with a target of 2021 has reached elimination status. The target can be achieved if there is a commitment of the local government, especially in financing. The number of malaria-cases in West-Seram is in line with the number of malaria-cases nationally, which tends to decrease. The number of malaria-cases nationally in 2010 was 465,764 cases with
an API of 1.96 “and decreased in 2014 to 252,027 cases with an API of 0.99”\(^{(12)}\).

The number of cases and deaths in the world (WHO), there was a decrease from 2000-2015 there was a decrease in cases by 60% and a decrease in deaths by 37\%\(^{(1)}\). The South Buru Health Office noted that 494 cases of malaria were positive in 2014 and API 6.86\%\(^{(5)}\). Sillehu et al. (2019) stated that the high and low cases of malaria in Buru Regency in closed and open community groups were related to the interaction of the host with agents due to agricultural land opening, high population mobility to endemic areas\(^{(10)}\). The malaria-elimination in West-seram will be maximal if there is support from all parties, across sectors and across programs and is supported by public awareness in participating in the success of malaria-elimination activities.

**Conclusion**

The malaria-prevention and eradication in West-Seram is carried out across sectors, inputs such as facilities and infrastructure are available as needed. The process in terms of implementation in the form of case finding. Increasing the capacity of human-resources in supporting malaria-elimination programs through active case detection education and training programs, supporting local governments in providing diagnostic and financing support facilities. Prevention program with the use of mosquito nets to avoid contact with mosquitoes. The availability of medicines is adequate for vector control.

**Conflict of Interest**-No

**Funding**-Authors

**Ethical Clearance**-Yes

**References**

Factors Related to Nurses’ Work Stress in the Toto Kabila Hospital

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Abstract

Stress at work can not be avoided, nurses are at risk of experiencing work stress. The purpose of this study is to find factors related to the nurse’s work stress. This type of research was observational analytic using a cross-sectional study design. The sample was 96 nurses working in the Toto Kabila hospital. Data were analyzed using chi-square tests. The marital status (p-value=0.001), length of work (p-value=0.001), working conditions (p-value=0.004), social support (p-value=0.010) had significant relationship with work stress. The results of this study are expected to be used as input and consideration for nurses at Toto Kabila Hospital to maximize how to deal with stress.

Keywords: work stress, marital status, length of work, working conditions, social support

Introduction

Nurses’ work routines that require speed and responsiveness to situations, especially nurses working in the emergency department, result in heavy work pressure resulting in work stress (distress). Work in the formal and informal sectors in government and non-government institutions has rules of working time, demands for good performance, and maximum productivity. This results in pressure on the workforce so that it can cause negative work stress.(1)

In the emergency room at all times, there are cases with varying degrees of emergencies that must be served immediately. Nurses as health workers who are always in the first contact with patients must always be fast, precise, and careful to prevent death and disability.(2)

According to WHO (2014), in many countries 8% of occupational diseases are depression. Research results in 2014 found 440,000 cases of work-related stress in the UK with an incidence of 1,380 cases per 100,000 workers experiencing work-related stress. A survey of nurses at Medan Haji Hospital found nurses’ work stress to have a negative impact (distress) 32% mild stress, 62% moderate and 6% severe.(1) The impact of work stress experienced by nurses is the low positive image of nurses.(3)

There are 35% of work-related stress is fatal and it is estimated that working days are lost by 43%. Based on a survey of West Australian health statistics it was stated that male workers lost approximately 50.8 working days and female workers lost approximately 58.5 working days.(4)

The national prevalence of mental-emotional stress prevalence is 6% and is not differentiated between workers and non-workers. The survey conducted stated that 64% of workers in Indonesia experienced an increase in stress compared to 2011. As much as 1.6% of the population of Jambi Province were estimated to experience mental-emotional disorders. Health problems related to work result in economic losses of 4-6% of GDP for most countries. Stress due to work is an important occupational health problem, which will result in a significant decrease in work productivity.
Utami (2015) states that public health does not only look at the community in general but also the working community because public health is a multidisciplinary science consisting of 7 disciplines, one of which is occupational health.\(^{(5)}\)

The survey at Toto Kabila District General Hospital showed that there were 111 nurses in 2015, 111 nurses in 2016 and 132 nurses in 2017. The survey obtained from the results of the number of patients in 2015 was 8,561 patients, in 2016 there were 9,782 patients, and in 2017 there were 9,891 patients. The division of nurses’ work with 3 work shifts, and divided by units namely ICU, IGD, Interna 1, Children, Neonatal Intensive Care Unit, puerperal midwifery, midwifery, Flamboyant / VIP, Surgery, and room Interna 2. Based on these data it is necessary to research work stress problems so that the formulation of this research problem is: what factors are related to nurse’s work stress? The purpose of this study: to find factors related to the nurse’s work stress.

### Method

The design of this research was cross-sectional. The study was conducted at the Toto Kabila Hospital, Bone Bonelango District, from February to March 2018. The population was all nurses who worked at Toto Kabila Hospital in 2018 (97 people). The sampling technique was total sampling.

The dependent variable was work stress and the independent variables were: age, marital status, length of work, working conditions and social support. Data were analyzed using Chi-square test.

### Findings

The characteristics of respondents such as age, sex, employment status, and education, the results are presented in the table 1.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-25</td>
<td>29</td>
<td>30.2</td>
</tr>
<tr>
<td>26-30</td>
<td>36</td>
<td>37.5</td>
</tr>
<tr>
<td>31-45</td>
<td>31</td>
<td>32.3</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>27</td>
<td>28.1</td>
</tr>
<tr>
<td>Female</td>
<td>69</td>
<td>71.9</td>
</tr>
<tr>
<td>Employment status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Civil servants</td>
<td>46</td>
<td>47.9</td>
</tr>
<tr>
<td>Honorary</td>
<td>50</td>
<td>52.1</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diploma III</td>
<td>57</td>
<td>59.4</td>
</tr>
<tr>
<td>Bachelor</td>
<td>39</td>
<td>40.6</td>
</tr>
</tbody>
</table>

The majority of age was 26-30 years old; the majority of gender was female; the majority of employment was honorary; and the majority of education was diploma III.
**Table 2. Factors associated with nurse’s work stress**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Works stress</th>
<th>Total</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Moderate</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>%</td>
<td>F</td>
</tr>
<tr>
<td><strong>Age (year)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤40</td>
<td>64</td>
<td>79.0</td>
<td>11</td>
</tr>
<tr>
<td>&gt;40</td>
<td>15</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>17</td>
<td>60.7</td>
<td>6</td>
</tr>
<tr>
<td>Married</td>
<td>62</td>
<td>91.2</td>
<td>5</td>
</tr>
<tr>
<td><strong>Length of work (year)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤5</td>
<td>22</td>
<td>66.7</td>
<td>5</td>
</tr>
<tr>
<td>&gt;5</td>
<td>57</td>
<td>90.5</td>
<td>6</td>
</tr>
<tr>
<td><strong>Working conditions</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less</td>
<td>26</td>
<td>72.2</td>
<td>9</td>
</tr>
<tr>
<td>Enough</td>
<td>53</td>
<td>88.3</td>
<td>2</td>
</tr>
<tr>
<td><strong>Social support</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less</td>
<td>50</td>
<td>82.0</td>
<td>10</td>
</tr>
<tr>
<td>Enough</td>
<td>29</td>
<td>82.9</td>
<td>1</td>
</tr>
</tbody>
</table>

**Discussion**

Age is the length of time of life or existing (since birth or held). Older employees tend to have a greater sense of attachment or commitment to the organization compared to the younger age so that it increases their loyalty to the organization.(7) Utami (2015) elaborates that in general the discussion of variables on individual characteristics is age, gender, and ethnicity. Age groups under 40 years have a high mortality rate. Several studies describe that health, illness, and treatment are age-related.(5),(8)

It was concluded that there was no significant relationship between the age of nurses and work stress. The results of this study are following research by Ibrahim et al. (2016) which states that workers under 40 years experience more stress than workers aged over 40 years. According to him, workers aged 40 years and over have good abilities in controlling work stress.(9)

The results of this study are in line with the results of research conducted by Mareta 2016 which was obtained from the results of data analysis using the Chi-square Test obtained p-value = 0.908, which means there is no significant relationship between the age of nurses with work stress in the nursing room of Dr. Soehadi Prijinegoro Hospital, Sragen. One research shows that employees who get married have fewer absences, experience lower turnover, and are more satisfied with their work than bachelor coworkers.

It was concluded that there was a significant relationship between the marital status of nurses with work stress. The results of this study are not in line with the results of research conducted by Mareta 2016 where obtained from the results of data analysis using the Chi-square Test obtained P-value = 0.444, which means there is no significant relationship between work time with work stress on nurses in the room inpatient Dr. Soegiri Hospital, Lamongan.(7)

Length of service (length of work) is an individual experience that will determine growth in employment and occupation, work period shows how long a person

...
works in each job and position.\(^{(7)}\) It was concluded that there was a significant relationship between the length of time the nurse worked with work stress. The results of this study are not by previous studies, Ibrahim et al (2016) the relationship of work tenure with work stress has a p-value = 0.70 (>0.05) which means that work tenure is not related to work stress. Workers who have long years of work experience less work stress, and fewer new workers who work stress. The assumption is that the longer a person works, the greater the responsibility he receives.\(^{(9)}\) Seniority determines the greater responsibility placed on workers. Yanto & Rezeki (2017) argue that nurses are young and have no experience, but are more energetic and energetic at work. The results of his research found that the age of the nurse together with the mentoring program had a significant effect on reducing nurses’ work stress.\(^{(10)}\)

The work period provides experience for the worker, the longer he works, the more experience he gets. Contradicts previous research. Research conducted by Mareta 2016 which was obtained from the results of data analysis using the Chi-square Test obtained p-value = 0.255, which means there is no significant relationship between work time and work stress on nurses in the inpatient Dr. Soegiri Hospital, Lamongan.\(^{(7)}\)

Social support is an act that is helpful by involving emotions, providing information, material assistance and positive assessment of individuals in dealing with their problems. It was concluded that there was a significant relationship between nurses’ social support and work stress. Working conditions can be a motivation for workers. Purwandari (2015) research results nurses who have good work motivation, are at mild work stress levels.\(^{(11)}\)

Fuada et al (2017) examined factors related to nurse stress, one of the factors that influence work stress is career development. Nurses who experience heavy work stress are dominated by nurses who develop poor careers. This means that social support in the work environment such as no career development is the cause of the emergence of work stress. The results of this study are not in line with the results of research conducted by Hany Ummu which obtained results using multiple regression analysis with the enter method with the results of the analysis obtained values R = 0.633, R² = 0.400, F = 39.050, P = 0.000, shows there is a significant relationship between stress and social support for nurses.\(^{(12)}\)

It was concluded that there was a significant relationship between the working conditions of nurses and work stress. Fajrillah (2016) high nurse work stress has an impact on low nurse performance.\(^{(13)}\) Working conditions are a series of conditions or working environment conditions of a company that is a place of work of the employees who work in the environment and what is meant here is a good working condition that is comfortable and supports workers to be able to carry out their activities well.\(^{(14)}\) Working conditions such as shift work and workload are factors that cause work stress. Previous studies found a significant relationship between work shift and work stress in nurses, due to perceived fatigue.\(^{(15)}\) Azizah & Nopti’s research (2019) shows that there is a relationship between workload and the stress level of nurses working in Tangerang District public hospitals in the emergency department.\(^{(16)}\) Elizar (2020) based on the results of his research found a relationship between workload and work stress.\(^{(17)}\)

The characteristics of the social environment become a source of stress in an organization because it can form interpersonal relationships between coworkers, superiors, and consumers that have the potential to trigger conflict.\(^{(18)}\) The results of this study are in line with the results of a study conducted by Mareouw in which the results obtained from the chi-square test obtained value = 0.001, which there is a significant relationship between working conditions with work stress on nurses in the medical emergency room at the Central Hospital Prof Dr. R.D. Kandou, Manado.

Conclusions

Factors related to nurses’ work stress at hospital Toto Kabila hospital are marital status, length of work, working conditions, social support.

Source of Funding: Authors

Conflict of Interest: No

Ethical Clearance: Yes

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microRNA as Potential Biomarker for Pediatric Tuberculosis?

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Abstract

The diagnosis of pediatric TB is based on history taking, clinical symptoms, physical examination and support. In recent years the role of microRNA (miRNA or miR) has become a concern for researchers as biomarkers of diagnosis and therapy in TB in adults and children. MicroRNA is a ribonucleic acid that does not encode proteins with 18-25 nucleotide transcripts that interact with gene targets and regulate mRNA expression. miRNA works with other regulatory elements such as transcription factors to control mRNA translation. More than 100 different miRNAs are expressed by immune system cells; they have the potential to broadly influence the molecular pathways that control the development and function of innate and adaptive immune response regulation. During TB infection, the innate immune response provides an initial defense mechanism against infection. It is well known that macrophages are the main stem cells for mycobacteria, survival in macrophages is determined by host-pathogen interactions. Several studies have shown that miRNA can be used as a biomarker and TB therapy agent because it is stable in plasma and other body fluids, difficult to degrade and excreted in the form of exosomes or micro vesicles. Other studies say miRNA is stable despite repeated exposure to heat, cold, acids, bases, and other extreme conditions. miRNA levels are reported to be increased in individuals with TB.

Keywords: pediatric tuberculosis; diagnosis; therapy; microRNA; biomarker

Introduction

Tuberculosis (TB) is a chronic infectious disease caused by infection with Mycobacterium tuberculosis (M.tb). This disease is the second leading cause of death in the world due to infection after HIV / AIDS¹,². It is estimated that around two billion people suffer from latent TB infection and cause new TB cases in 9.2 million people and deaths in 1.7 million people in the world. About 5-10% of people who suffer from latent TB infection will become active TB in the first five years after becoming infected with TB germs³,⁴. In 2015 there were 10.4 million new TB cases worldwide, 10% of which were Pediatric TB⁵.

The diagnosis of pediatric TB is based on history taking, clinical symptoms, physical examination and support. However, children infected with TB often show typical symptoms⁶,⁷. Tuberculin testing based on cellular immunity is considered impractical because it requires a minimum of two diagnostic visits. Tuberculin test can be affected by various conditions so that it can produce false positives or false negatives⁶–⁸. Other investigations that are believed to be used in the diagnosis of TB include Interferon Gamma Release Assay (IGRA). The use of IGRA for the diagnosis of TB in children is still limited due to the high cost of examinations and the lack of examination tools and techniques. This examination still cannot distinguish active and latent TB infections⁹,¹⁰.

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The definitive diagnosis of TB is made by finding TB germs on direct smear examination and culture which is a gold standard examination. But a definitive diagnosis in children is difficult to obtain because of the small number of germs (pausibasiler), the child is difficult to expel phlegm, the location of germs in the parenchyma area far from the bronchus. Indonesia has developed a TB scoring system for children and is still being applied to help establish a diagnosis of TB in children\textsuperscript{(7,8,10,11)}.

In recent years the role of miRNA has become a concern for researchers as biomarkers of diagnosis and therapy in TB in adults and children. Identification/profiling studies of miRNA in TB with microarray profiling have been carried out in countries. There are significant differences between levels of miRNA expression in pediatric TB patients compared with healthy children and have significant diagnostic values. This suggests that miRNA can be one of the tools to diagnose TB in children that needs to be developed\textsuperscript{(9,12-20)}.

**microRNA**

microRNA is a ribonucleic acid that does not encode proteins with 18-25 nucleotide final transcripts that interact with gene targets and regulate mRNA expression. miRNA works with other regulatory elements such as transcription factors to control mRNA translation. Most miRNAs are encoded in parts of the introns that used to be considered non-coding regions. The miRNA genes are distributed in the genome and are estimated to make up 2-5% of the human genes. miRNA is often expressed as a polysistronic transcript. One miRNA can have many target mRNAs so it is estimated that more than 1/3 of human genes are regulated by miRNA\textsuperscript{(13,20,21)}.

More than 100 different miRNAs are expressed by immune system cells; they have the potential to broadly influence the molecular pathways that control the development and function of innate and adaptive immune response regulation. Apart from impaired immune function, miRNA is also known to play a role in infection. microRNA plays an important role in cell processes such as cell differentiation, cell cycle, embryonic development, apoptosis and associated with infectious diseases including TB\textsuperscript{(22,23)}. Research has shown that stable miRNA serum faces repeated freezing and thawing as well as heat, acid and base conditions and other extreme conditions. This has the potential to be a useful biomarker for disease diagnosis, the effects of therapy and prognosis\textsuperscript{(20)}.

**microRNA Biogenesis**

microRNA is a small, encoded RNA molecule that regulates several biological processes by interfering with mRNA translation. miRNA biosynthesis through a process involving several proteins and enzymes found in the nucleus and cytoplasm. miRNA biosynthesis originates from the miRNA gene in the nucleus where the main miRNA undergoes transcription as a large double-chain primary transcript called pri-miRNA by RNA polymerase II. Pri-miRNA folded into structures such as hairpins undergo polyadenylation and capped. The Drosha RNAse type III enzyme converts this precursor into a double chain miRNA precursor from 60 to 100-nt hairpin known as pre-miRNA. Pre-miRNA consists of a local stem-loop structure that encodes the miRNA sequence that is exported from the nucleus to the cytoplasm by exportin 5\textsuperscript{(9,23)}.

In the cytoplasm, pre-miRNA is further processed by Dicer RNAse III to become duplex miRNA. This unstable duplex consists of guide strand (miRNA) and passenger strand (miRNA *). The miRNA strand guide chain will become mature miRNA, where miRNA * is degraded. Mature miRNA is facilitated by Argonaut protein incorporated into RNA-induced silencing complex (RISC), which recognizes specific miRNA targets and triggers post-transcriptional silencing genes to regulate protein expression, target cleavage mRNAs, suppress / inhibit translation processes, and deadenylate mRNA\textsuperscript{(9,22)}.

**The Role of microRNA in Tuberculosis**

Studies show that different miRNAs play a major role in the process of cell differentiation involved in protection against bacterial infections, such as differentiation and function of immune cells, control of chemokine production and regulation of the immune response. miRNA regulates gene expression by targeting mRNA3'-regions that are not translated, modifying translation and / or degradation. During TB infection, the innate immune response provides an initial defense mechanism against infection. It is well known that macrophages are the main stem cells for mycobacteria, survival in macrophages is determined by host-pathogen interactions\textsuperscript{(24-31)}.

Study found disturbed gene expression profiles in macrophages and NK cells from active TB, latent TB and healthy controls that appeared to be regulated by miRNA. Some miRNAs play a role in the regulation of
T cell differentiation and function. Research shows that miRNA plays an important role in regulating the innate functions of macrophages, DC and NK cells (12,32). Hundreds of miRNA encoded in the human genome and thousands of mRNAs have been shown to be involved in cell development, differentiation, proliferation, apoptosis, DNA methylation, DNA repair and regulating anti-inflammatory or pro-inflammatory stimuli (33).

Research by Zhou found 14 miRNAs that are very important in pediatric TB. RT-qPCR validates that miRNA-1, miRNA-155, miRNA-31, miRNA-146a, miRNA-10a, miRNA-125b and miRNA-150 are downregulated while miRNA-29 experiences upregulation in children with TB compared to children with TB not infected (20).

Modulating the expression of miRNA-125b, which, in turn, will reduce the level of TNF-α and other major cytokines for controlling M. tb. The targets of miRNA-132 and miRNA-26a show a mechanism by which M. tb can limit macrophage responses to IFN-γ by interfering with the expression of host miRNA (35). A study reported that miRNA-21 inhibits the expression of proinflammatory cytokines and increases the production of anti-inflammatory cytokines, IL-10. miRNA-21 is upregulated in un-sensitized DC and macrophages via the TLR/Erk/NF-κB pathway and also regulated following macrophages together with M.Tb which secretes 6 kDa protein (ESAT-6). Inhibition of IL-12 expression by targeting 3′UTR IL-12 mRNA directly and suppressing Th1 response (2,36).

Clinical and in vitro studies note that miRNA-29 is over-expressed after M.Tb infection in several types of human cells. miRNA-29 suppresses the immune response to M.Tb by downregulating IFN-γ. Besides targeting IFN-γ mRNA 3′UTR, miRNA-29 forms an IFN-R mRNA relationship with Argonaut 2 protein (Ago2) to form RNA-induced silencing complexes and subsequently suppress IFN-γ expression post-transcriptionally. Several studies indicate that miRNA-29 also targets anti-apoptosis B-cell lymphoma 2 (Bcl-2) proteins and regulates the apoptotic pathway in immune cells (16,18,37,38). Understanding the expression patterns and regulation of miRNA in active TB and latent TB infection opens the possibility that miRNA can be used as a potential diagnostic marker candidate for TB.

**microRNA as a Biomarker in Tuberculosis**

Early diagnosis is important in efforts to control or effectively treat TB. The heterogeneous clinical presentation of M.tb infection (active TB, latent asymptomatic TB, pulmonary TB, and extra pulmonary TB) is a reason for the development of diagnostic biomarkers. It aims to improve the quality of diagnostic papal bacillary TB or TB cases that are difficult to ascertain for example in children, individuals with HIV, extra pulmonary cases.

The mechanism of LTBI and its transition to active TB is still unclear. Many studies provide evidence that this transition arises if cell-mediated immunity fails. Previous studies have shown that gene expression profile is disrupted in macrophages and NK cells from active tuberculosis and LTBI is thought to have a major role in controlling miRNA expression and describing significant markers for knowing and diagnosing active tuberculosis and LTBI (39).

Sputum, serum, plasma, or other body fluid specimens can be used for non-invasive miRNA analysis, in addition the level of miRNA expression seems stable and can be reproduced in serum, which makes them a potential marker for disease diagnosis so that miRNA can be considered a biomarker which is ideal for TB disease diagnosis (40).

Overall previous research revealed the role of miRNA in the immune and inflammatory response to TB. There is evidence that circulating miRNA exerts biological functions as part of intercellular communication and can be used as biomarkers for human disease. The attractiveness of using plasma miRNA in clinical applications is very high, because the separation and storage of plasma or serum samples is already a clinical routine in TB endemic countries (19).

Several studies have shown that miRNA can be used as a biomarker and TB therapy agent because it is stable in plasma and other body fluids, difficult to degrade and excreted in the form of exosomes or micro vesicles. Other studies say miRNA is stable despite repeated exposure to heat, cold, acids, bases, and other extreme conditions. MiRNA levels are reported to be increased in individuals with TB (14,17). Studies conducted on children show that there are significant differences between miRNA levels in children with TB compared to healthy children and have significant diagnostic values. This shows that miRNA can be one of the tools to diagnose TB in children that needs to be developed (15,18).
Additional Informations

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References


The Effect of Acceptance and Commitment Therapy on The Quality of Life of Post Stroke Patients in Aloei Saboe Hospital

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Abstract

Quality of life is a global concept that emphasizes dimensions of health status including finance, housing, and work. The quality of life of post-stroke sufferers can experience disturbances or obstacles due to physical disabilities, cognition, psychological and social disorders. The purpose of this study was to determine the effect of ACT interventions on changes in the quality of life of post-stroke patients at Aloe Saboe Hospital. The method used in this research is quasi-experimental research method pre-posttest with the control group. The sampling technique used was the purposive sampling technique. The number of samples studied was 60 respondents divided into the experimental group (ACT Therapy and health education) and the control group (health education). The variables used are independent variables namely ACT therapy and the dependent variable is the quality of life of patients after stroke. Based on the results of the analysis, there is an effect of ACT intervention on improving quality of life in post-stroke patients as evidenced by the results of \textit{t} count = -9.015, then the Significant value is 0.000. This shows that the value of \textit{p} < 0.05 then Ho is rejected, so the research hypothesis is proven that there is an effect of ACT therapy on the quality of life of post-stroke patients at Aloei Saboe Hospital, Gorontalo City.

\textit{Keywords}: ACT; post-stroke; quality of life; therapy; acceptance; commitment

Introduction

Stroke is a disease in the brain in the form of impaired local or global nerve function that appears suddenly, progressive, and fast. Impaired nerve function in stroke is caused by nontraumatic brain blood circulation disorders. Symptoms that cause paralysis of the face and limbs, speech is not smooth and impaired vision. Stroke can be interpreted as any damage to the brain of the central nervous system caused by abnormalities/abnormalities of blood vessels. The term stroke is always used when the symptoms occur acutely, while the term cerebrovascular disease is used more generally and is not related to the time of brain damage. The World Health Organization (WHO) states that stroke is a functional impairment of vocal and global brain affecting the quality of life of sufferers.\textsuperscript{(1)}

The incidence of stroke in Indonesia has been declining over time but the prevalence of rates has increased. This shows that the number of old stroke cases increases each year, thus meaning more people with disabilities due to stroke so that the rehabilitation function is more important. Stroke recovery rates are still low, as many as 15-30\% sufferers will experience paralysis or permanent disability, loss of voice or memory and various other consequences. About 25\% of stroke patients die within the first year after a stroke and 14-15\% experienced a second stroke in the same year after a first stroke.\textsuperscript{(2)}

According to the 2013 Basic Health Research (Riskesdas) data, the prevalence of stroke in Indonesia is 12.1 per 1,000 population. That number is up compared to Riskesdas 2007 which amounted to 8.3 percent. Judging from its characteristics, many strokes are experienced by the elderly, have low education, and live in cities. Based
on data from Aloe Saboe Hospital, Gorontalo City, it was found that there were 1560 stroke sufferers in nerve poly consisting of 739 Hemorrhagic stroke sufferers and 821 Non-Hemorrhagic Stroke sufferers.

Acceptance and commitment therapy (ACT and Commitment Therapy / ACT), is one of the most popular therapies today and is considered more flexible and more effective in handling various cases. This therapy teaches patients to accept thoughts that are distracting and considered unpleasant by placing themselves following their values so that patients will accept existing conditions.\(^{(3)}\)

Acceptance and commitment have a huge impact on the development of patients with anxiety to be better. Commitment means an agreement (attachment) to do something. ACT is very effective in creating acceptance, attention and being more open in developing the capabilities of depression, anxiety, drug abuse, chronic pain, schizophrenia patients and is very effective as a model of self-training. Therefore ACT is expected to improve the quality of life in patients with post-stroke.

The results of Bays\(^{(4)}\) in the United States showed a decrease in the quality of life of post-stroke patients including daily activities, communication patterns, social activities, work, rest and recreation. Declining quality of life can affect the lives of sufferers and caregivers. Therefore the family also plays a role in improving the quality of life of sufferers. According to Nurrohma\(^{(5)}\), quality of life as a center for health promotion, quality of life is based on three areas of human life that are important dimensions in human experience, namely: Being, Belonging and Becoming. These three things occur due to interactions between a person and his environment.

Based on the description that has been explained, the researcher is interested in researching with the title the effect of acceptance and commitment therapy on the quality of life of patients after stroke in Aloe Saboe Hospital, Gorontalo City.

**Method**

This research was conducted at the Regional General Hospital (RSUD) Aloe Saboe Gorontalo City. This type of research is a quasi-experimental research pre-post test with a control group. This research was a type of research that tests an intervention in a group of subjects with a comparison group. The intervention that was tested was the provision of ACT, which in the implementation stage was modified by researchers using a spiritual approach. The study population was patients who visited the Aloe Saboe Hospital in Gorontalo City, amounting to 60 patients who were determined by the purposive side technique. The sample used was by the inclusion criteria set by the researchers, namely a) outpatients diagnosed after stroke; b) experiencing changes in quality of life; c) the level of consciousness of compos mentis; d) cooperative and willing to be respondents given ACT therapy. Data were collected using a demographic data questionnaire, namely the WHOQOL-BREF quality of life questionnaire, which contained aspects of quality of life that included physical dimensions, psychological dimensions, social relations dimensions, and environmental dimensions. Besides, an examination of risk factors, namely blood pressure, and cholesterol to see the effect on the quality of life. Data were analyzed by paired sample t-test to prove the research hypothesis by looking at differences in quality of life in the intervention and control groups before and after the intervention. To determine the homogeneity of variables between the intervention group and the control group, the equality test was conducted. The independent sample t-test was used to determine the differences between the two groups. A multivariate analysis process was carried out to prove the relationship between the characteristics of post-stroke patients with the quality of life.

**Findings**

The results of the study are presented in the table 1. Based on the table 1, it shows that the characteristics of respondents consisted of gender, age, last education, and last occupation.
Table 1. Characteristics of respondents

<table>
<thead>
<tr>
<th>Characteristics of respondents</th>
<th>Total</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experiment</td>
<td>%</td>
<td>Control</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Male</td>
<td>16</td>
<td>53.3</td>
<td>13</td>
</tr>
<tr>
<td>• Female</td>
<td>14</td>
<td>46.7</td>
<td>17</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Adult (16-45)</td>
<td>5</td>
<td>16.7</td>
<td>8</td>
</tr>
<tr>
<td>• Elderly (46-&gt;65)</td>
<td>25</td>
<td>83.3</td>
<td>22</td>
</tr>
<tr>
<td>• Last Education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• High</td>
<td>10</td>
<td>33.3</td>
<td>17</td>
</tr>
<tr>
<td>• Low</td>
<td>20</td>
<td>66.7</td>
<td>13</td>
</tr>
<tr>
<td>• Last Occupation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Employee</td>
<td>19</td>
<td>63.3</td>
<td>21</td>
</tr>
<tr>
<td>• Unemployed</td>
<td>11</td>
<td>36.7</td>
<td>9</td>
</tr>
</tbody>
</table>

Based on table 2, there is an influence of cholesterol risk factors on quality of life before and after health care and ACT therapy in the treatment group with each value p-value = 0.000 (<0.05). and on hypertension risk factors there is no influence on the quality of life before the intervention with a p-value of 0.961, and after the intervention, there is an influence of hypertension risk factors on quality of life with a p-value = 0.000.

Table 2. Effect of risk factors on quality of life before and after health education and ACT therapy in the treatment group

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Quality of life</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Good</td>
<td>Poor</td>
</tr>
<tr>
<td>Cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Normal</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>• Abnormal</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>After</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Normal</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>• Abnormal</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Normal</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>• Abnormal</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>After</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Normal</td>
<td>27</td>
<td>0</td>
</tr>
<tr>
<td>• Abnormal</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

Based on the table 3 there was no influence of cholesterol risk factors on quality of life before the provision of health education in the control group with a p-value = 0.0196 (>0.05) as well as after the intervention did not affect with a p-value = 0.0196 (>0.05). For risk factors for hypertension also did not have a good effect before the
intervention with p-value = 0.0196 (>0.05) and after the intervention with p-value = 0.0196 (>0.05).

Table 3. Effects of risk factors on quality of life before and after health education and ACT therapy in the control group

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Quality of life</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Good</td>
<td>Poor</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Normal</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Abnormal</td>
<td>7</td>
</tr>
<tr>
<td>After</td>
<td>Normal</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Abnormal</td>
<td>8</td>
</tr>
</tbody>
</table>

Based on the table 4, there was an effect of providing health education and ACT therapy in the experimental group with a p-value of 0.000 (<0.05). In the control group that was only given a health education intervention p-value = 0.139 (>0.05) then there was no effect of the intervention on quality of life.

Table 4. Influence of intervention on group experiments and control

<table>
<thead>
<tr>
<th>Group</th>
<th>Quality of Life</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Good</td>
<td>Poor</td>
</tr>
<tr>
<td>Experiment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Health Education and ACT)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>1</td>
<td>29</td>
</tr>
<tr>
<td>After</td>
<td>26</td>
<td>4</td>
</tr>
<tr>
<td>Control (Health Education)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>After</td>
<td>23</td>
<td>7</td>
</tr>
</tbody>
</table>

Discussion

According to Wijaya & Putri\(^6\), the incidence of stroke increases with age. Data reported by the American Heart Association in the Heart Disease and Stroke Statistics-2011 Update that young stroke sufferers between 20-45 years have increased dramatically in recent years. In 2009 the age range of ischemic stroke patients was between 20-60 years, with an average age of 58.8 years. Meanwhile, in 2010 the age range of ischemic stroke patients was between 24-90 years with an average age of 48 years. This shows that both in Indonesia and in the world, stroke has attacked many productive ages and even children.\(^7\)

Based on the results of the study, the gender frequency distribution of stroke clients in this study was more dominated by men. This is in line with the theory that men are more often found to suffer a stroke than women.\(^6\) Bowman also revealed that the incidence of stroke in men was slightly higher than in women, this difference occurred probably related to the increased incidence of hypertension and diabetes in the group.\(^9\) The study is in line with the theory that men are more at risk of stroke than women, with a percentage of 20 percent higher in men than women. But after a woman turns 55, when estrogen levels decrease due to menopause, the risk is actually higher than men.\(^4\)

The results of the analysis of the relationship between work and the incidence of stroke show that most respondents have jobs. Researcher’s assumption, there is a significant relationship between work and the incidence of stroke. Work is a risk factor for stroke. This might be caused by the relationship between work and one’s stress level. A large workload, the salary that is not as expected, and pressure from superiors can trigger stress and be a risk factor for stroke. Stressful conditions can produce the hormones cortisol and adrenaline which contribute to the process of atherosclerosis. This happens because the two hormones increase the platelet count and the production of cholesterol which can damage cells lining the arteries making it easier for fat tissue to be buried in the artery walls.\(^5\)

The analysis showed that cholesterol risk factors before and after health education and ACT intervention had a significant effect on improving the quality of life of respondents. Meanwhile, the risk of hypertension after being given health education and ACT Interventions has a significant effect on improving the quality of life of respondents. Researcher’s assumption that cholesterol
and hypertension are risk factors that affect the quality of life of respondents after a stroke due to increased body cholesterol can cause atherosclerosis and the formation of fat embolism so that blood flow slows into the brain, brain perfusion decreases.\(^{(6)}\)

Based on the results of the study, it shows that before being given ACT therapy the average quality of life score in the intervention group had a mean value of 67.0 with the bad category. And after the intervention, it becomes 81.0 with a good category. The researcher’s assumption, this shows an increase in the quality of life score that shows the increased quality of life experienced by respondents. ACT therapy with a spiritual approach to quality of life can be said to be influential because it gets the result of \(t\) count = -9.015, then significant is 0.000. an important role in determining abnormalities. Neurons or nerve cells are the basic units of structure and function in the nervous system. Neurons are communicators that send information between the body and brain.\(^{(7)}\)

Emotional problems are common, anxiety and depression reactions are left-brain damage reactions while the damage to the right brain often causes a strange feeling or different from the situation at hand. This is experienced by many stroke patients. They cannot assess precisely the damage done. As a result, depression arises as a symptom that often accompanies this disease. Thus, psychospiritual assistance is very helpful in dealing with depression in dealing with this disease.\(^{(8)}\) In implementing ACT therapy, researchers modify the therapy given at each stage with a spiritual approach.

At the ACT therapy stage which is the stage of “practicing accepting selected events and values”, the spiritual approach taken is to understand that illness, recovery, and treatment are provisions and decrees from God. Similarly, at the ACT stage “commit and prevent recurrence”, it was conveyed that prevention and treatment efforts can be done by surrendering and getting closer to God.

Some neurologists examine the existence of a god spot (God’s point) in the human brain. This spiritual center is located between the connections of nerves in the temporal lobe of the cerebrum. Based on observations using emission topographic observations and positrons, the area reacts when the research subjects discuss topics with religion. Worship, regardless of religion, will lead us to focus attention in the mind with the aim of contemplation. The activity, besides having a spiritual function, also has a health function. In the spiritual function, the prefrontal cortex will work actively. This area is associated with positive emotions and makes the cerebral cortex thicken, due to the growth of glial cells supporting neuron cells and neurons that show positive reactions by forming relationships between neurons or synapses. This synapse plays a role in the delivery of information to and from the brain.\(^{(9)}\)

Based on the results of the study, it shows that before being given a health education intervention, the average quality of life score in the control group had a mean value of 77.13 with a bad category. Then after the intervention, it becomes 78.80 with a good category. In this study, health education interventions on quality of life can be said to not affect because the results obtained \(t\) count = -1.520, then significant is 0.139. The researchers assume that ACT therapy with a spiritual approach is more effective in improving the quality of life of post-stroke respondents compared to health education in the control group because there is no significant difference between the quality of life scores before and after health education interventions are given. The control group only experienced an increase in the quality of life score of 1.67.

**Conclusion**

Based on the study it was concluded that the difference in the response to improving the quality of life in post-stroke patients in the intervention group with the control group, where after the intervention of respondents the ACT treatment group the average quality of life score increased to 81.0 while the improvement in the quality of life of the control group of respondents after being given health education became 78.8 this can be interpreted that the improvement in the quality of life of patients after stroke in the intervention group was higher than in the control group.

**Conflict of Interest**- No

**Source of Funding**- Authors

**Ethical Clearance**- Yes

**References**


Determinant Factors on Active and Latent Tuberculosis among Children in Surabaya

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Abstract

Background: Pediatric tuberculosis (TB) is a major health problem in the world and Indonesia. The difficulty of diagnosis is a major factor in eradicating TB in children. Active and latent tuberculosis in children often shows no symptoms so it is difficult to diagnose and affect the treatment. Several determinant factors need to be assessed with the hope of optimal handling of TB in children. Objective: To analyze the determinant factors on active and latent tuberculosis among children in Surabaya. Methods: A case control study was done with 25 cases with TB divided into active TB, Latent TB. Pulmonary tuberculosis was diagnosed using the Indonesian Pediatric Tuberculosis Scoring System. Determinant factors for tuberculosis development were history of contact with a TB patient, age, BCG immunization, knowledge, socioeconomic status and overcrowding living condition. Analysis was done using Fisher’s Exact and Mann-Whitney test. Findings: The characteristics of the study subjects consisting of 16 active TB children and 9 latent TB children. In the group of active TB children the largest population is girls (11 children), whereas in the latent TB group are boys (8 children). There was a significant difference between groups of active TB children and latent TB children (p = 0.000 <0.05). The other factors are showing nothing different in the both of group TB. Conclusion: The most significant determinant factor in active TB and latent TB in children is the appearance infiltrate of chest X-ray where active TB shows filtration and normal appearance in latent TB.

Keywords: Tuberculosis; children; active; latent; determinant factors.

Introduction

Tuberculosis (TB) is a chronic infectious disease caused by infection with Mycobacterium tuberculosis (M.Tb). This disease is the second leading cause of death in the world due to infection after HIV / AIDS. In Indonesia, pulmonary TB is still one of the main causes of morbidity and mortality in children¹,². The highest TB cases were found in the provinces of West Java, East Java and Central Java with the number of cases from the three provinces reaching 38% of all new TB cases in Indonesia³.

It is estimated that around two billion people suffer from latent TB infection and cause new TB cases in 9.2 million people and deaths in 1.7 million people in the world. About 5-10% of people who suffer from latent TB infection will become active TB in the first five years after becoming infected with TB bacterias. Children with latent TB infection are more likely to develop into a serious disease than adults⁴,⁵.

The diagnosis of paediatric TB is based on history taking, clinical symptoms, physical examination and support. History of contact, especially with active adult TB sufferers accompanied by a collection of clinical symptoms of children suspected of TB including coughing > 3 weeks, weight loss or not rising for no
apparent reason despite having received adequate nutritional treatment, prolonged or recurring fever for no apparent reason > 2 weeks, anorexia and inactivity. However, children infected with TB often show typical symptoms\(^{(6,7)}\). Chest radiographs to establish a TB diagnosis in children are not typical except in miliary TB. Tuberculin test based on cellular immunity is considered impractical because it requires a minimum of two diagnostic visits. Tuberculin test can be affected by various conditions so that it can produce false positives or false negatives\(^{(3,6,7)}\).

The problem of TB in children is caused by several factors, including limited data on active TB and latent TB infection in children and difficulty in establishing a diagnosis that affects the management of TB therapy in children. The definitive diagnosis of TB is made by finding M. Tb on direct smear examination and / or culture which is a gold standard examination. But a definitive diagnosis in children is difficult to obtain because of the small number of bacteria (pausibasiler), the child is difficult to expel sputum, the location of bacteria in the parenchyma area far from the bronchi\(^{(3,7,8)}\).

Indonesia has developed a TB scoring system for children and is still being applied to help establish a diagnosis of TB in children. Constraints in the scoring system include parameters used such as tuberculin test and chest X-ray not always available at primary health facilities\(^{(3,9,10)}\). This study aims to analyze the determinant factors that influence TB disease in children infected with Mycobacterium tuberculosis so that prevention can be done so as not to become ill with TB.

**Method**

Observational research with case-control design was conducted at the polyclinic and inpatient at Perak Timur Primary Health Care and the Lung Hospital in Surabaya in the city in October until Desember 2019.

Sample of study were pediatric patients aged 0 - <18 years who suffer from active TB and latent TB who examined themselves at the Perak Timur Primary Health Care and Surabaya Lung Hospital in the period October-Desember 2019. Inclusion criterion includes children aged 0 - <18 years, newly diagnosed pulmonary TB active or latent TB, has a history of contact with adult TB with positive microbiology examination, is willing to be the subject of research, that is stated by signing the consent sheet by the child’s parents. Meanwhile, the exclusion factors are children with congenital diseases, children with other chronic illnesses such as diabetes, malignancy, kidney disease, liver disease, blood diseases, allergies, malaria, and immunocompromised diseases such as HIV, children who have received TB treatment, children who are receiving immunosuppression therapy. Calculation of sample size in research uses consecutive sampling based on the number of patients available during the planned study period.

History and physical examination, nutritional status, chest X-ray and lateral position were performed. A positive tuberculin test showed TB infection. The diagnosis of tuberculosis was made by the scoring method. The scoring system for pediatric tuberculosis diagnosis included TB contact, tuberculin testing, nutritional status, fever ≥2 weeks without apparent cause, cough ≥3 weeks, enlarged lymph nodes, swollen joints / bones, chest X-Ray. The TB scoring system based on interviews conducted by researchers includes positive smear contacts and TB symptoms as well as tracking of risk factors using a questionnaire. This study was approved by the Medical Research Ethics Committee of the Faculty of Medicine, Wijaya Kusuma Surabaya University in 2019. Data analysed with Fisher’s Exact and Mann-Whitney with SPSS 20.0 (SPSS.Inc., Chicago, IL). Significant level was reached when p <0.05.

**Findings**

Table 1 shows the characteristics of the study subjects consisting of 16 active TB children and 9 latent TB children. In the group of active TB children the largest population is girls (11 children), whereas in the latent TB group are boys (8 children). The distribution of maternal education data in the group of active TB children showed that the most mothers with a high school education level were 12 people (75%). In the group of latent TB children, there were 7 mothers with the most recent education graduating from high school (77.8%). Whereas in the most types of father’s work was private sector which were 18 people (18%) in the active TB children group and there are 9 people (100%) in the latent TB children group.

Likewise in the educational characteristics of most fathers graduating from high school there were 12 people (75%) in the group of active TB children and there were 6 people (66.7%) in the group of latent TB children. Whereas in the most types of father’s work was private sector which were 18 people (18%) in the group of active TB children and employees/labourer’s/migrant
workers were 6 people (66.7%) in the group of latent TB children.

Table 1. Characteristics of respondents

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TB Active (n=16)</td>
<td>TB latent (n=9)</td>
<td></td>
</tr>
<tr>
<td>Gender :</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Boy</td>
<td>5 (31.3)</td>
<td>8 (88.9)</td>
<td></td>
</tr>
<tr>
<td>Girl</td>
<td>11 (68.8)</td>
<td>1 (11.1)</td>
<td></td>
</tr>
<tr>
<td>Mother’s Education :</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>0 (0)</td>
<td>2 (22.2)</td>
<td></td>
</tr>
<tr>
<td>Junior</td>
<td>3 (18.8)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Senior</td>
<td>12 (75)</td>
<td>7 (77.8)</td>
<td></td>
</tr>
<tr>
<td>Bachelor</td>
<td>1 (6.2)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Mother’s Occupation:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Housewife</td>
<td>15 (93.8)</td>
<td>9 (100)</td>
<td></td>
</tr>
<tr>
<td>Working</td>
<td>1 (6.3)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Father’s Education:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>3 (18.8)</td>
<td>2 (22.2)</td>
<td></td>
</tr>
<tr>
<td>Junior</td>
<td>1 (6.2)</td>
<td>1 (11.1)</td>
<td></td>
</tr>
<tr>
<td>Senior</td>
<td>12 (75)</td>
<td>6 (66.7)</td>
<td></td>
</tr>
<tr>
<td>Father’s Education:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Private Sectors</td>
<td>8 (50)</td>
<td>2 (22.2)</td>
<td></td>
</tr>
<tr>
<td>employees/labourer’s/migrant</td>
<td>5 (31.2)</td>
<td>6 (66.7)</td>
<td></td>
</tr>
<tr>
<td>Driver</td>
<td>3 (18.8)</td>
<td>1 (11.1)</td>
<td></td>
</tr>
</tbody>
</table>

Nutritional status in active TB children group were 13 children (81.2%) with good nutrition while in the most latent TB children group also in good nutrition (6 children (66.7%)). In the active TB children group it was found that there were 11 children (68.7%) showing weight loss symptoms and in latent TB children there were 4 children (44.4%) showing weight loss symptoms and there were 5 children (55.6%) showing no symptoms. MTX/Tuberculin test showed positive all in the group of active TB children (16 children) and in the latent TB children group (9 children). Likewise, the BCG vaccine for all children has had a BCG vaccine, both in the active TB children group (16 children) and in the latent TB children group (9 children). In the group of active TB children showed contact TB was found there were 15 children (93.8%) and in the group of latent TB children found that there were all TB contacts which were 9 children (100%).
Table 2. Characteristics internal factors respondents

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group</th>
<th>TB Active (n=16)</th>
<th>TB latent (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Nutrition Status:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>13 (81.2)</td>
<td>6 (66.7)</td>
<td></td>
</tr>
<tr>
<td>Less</td>
<td>3 (18.8)</td>
<td>2 (22.2)</td>
<td></td>
</tr>
<tr>
<td>Under</td>
<td>0 (0)</td>
<td>1 (11.1)</td>
<td></td>
</tr>
<tr>
<td>Symptoms :</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight loss</td>
<td>11 (68.7)</td>
<td>4 (44.4)</td>
<td></td>
</tr>
<tr>
<td>Cough</td>
<td>3 (18.8)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>2 (12.5)</td>
<td>5 (55.6)</td>
<td></td>
</tr>
<tr>
<td>MTX/Tuberculin Test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>16 (100)</td>
<td>9 (100)</td>
<td></td>
</tr>
<tr>
<td>BCG Vaccinee:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>16 (100)</td>
<td>9 (100)</td>
<td></td>
</tr>
<tr>
<td>Contact to TB :</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Yes</td>
<td>15 (93.8)</td>
<td>9 (100)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1 (6.2)</td>
<td>0 (0)</td>
<td></td>
</tr>
</tbody>
</table>

Fisher’s Exact test showed chest X-ray had a significant difference between active TB children and latent TB children (p = 0.000 <0.05). It was shown that there were 16 children (100%) in X-foto showing TB / infiltrates in the group of active TB children and there were 9 children (100%) in Chest X-ray showing normal in the group of latent TB children. (Table 3)

However, in smear or fast molecular examination there were 10 children (62.5%) negative in the active TB children group and 9 children (100%) in the latent TB children group. This shows that there was no significant difference in microbiology or fast molecular examination of the two groups (p = 0.057> 0.05).

Table 3. Characteristics of X Ray study samples

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group</th>
<th>TB Active (n=16)</th>
<th>TB latent (n=9)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chest X-Ray</td>
<td></td>
<td></td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>TB/ Infiltrate</td>
<td>16 (100)</td>
<td>0 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>normal</td>
<td>0 (0)</td>
<td>9 (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microbiology examination</td>
<td></td>
<td></td>
<td></td>
<td>0.057</td>
</tr>
<tr>
<td>Positive</td>
<td>6 (37.5)</td>
<td>0 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>10 (62.5)</td>
<td>9 (100)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Note: If the p-value < 0.05 means it is significant and if the p-value > 0.05 means no significant difference.

Seen in the free sample t test results in Table 4 shows there is no difference in the age of the child (p = 0.175 > 0.05) between children in the active TB group with the mean ± standard deviation of 62.4 ± 36.4 months with the average latent TB group of children ± standard deviation ie 85.6 ± 45.1 months.

Likewise, maternal age showed no significant difference (p = 0.262 > 0.05) between mothers in the active TB children group (30.56 ± 4.29 years) and mothers in the latent TB children group (33.44 ± 8.38 years). The two mean ages of the two groups were almost the same. This means that at the age of the mother has also shown homogeneous.

### Table 4. Age characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TB Active (n=16)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>Children (Month)</td>
<td>62.4±36.4</td>
<td>85.6±45.1</td>
</tr>
<tr>
<td>Mother (Year)</td>
<td>30.56±4.29</td>
<td>33.44±8.38</td>
</tr>
<tr>
<td>Father (Year)</td>
<td>33.81±6.84</td>
<td>37.11±8.11</td>
</tr>
</tbody>
</table>

Legend: If the p-value < 0.05 means there is a significant difference and if the p-value > 0.05 means there is no significant difference.

* T sample test results are free

** Mann-Whitney test results

Using the Mann-Whitney test showed no significant difference (p = 0.391 > 0.05) of the father’s age between the fathers of the active TB children group (33.81 ± 6.84 years) and the fathers of the latent TB children group (37.11 ± 8.11 years). The two mean ages of the two groups did not differ greatly. This means that at the age of the father also showed homogeneity.

**Discussion**

A factor that significantly indicates the occurrence of TB in both children with active TB and latent TB is chest x-ray images showing infiltrate. Significant differences were shown in chest X-ray images in the two groups, where the active TB group showed the presence of inflammation and the latent TB did not look normal. In another study it was stated that primary tuberculosis demonstrates radiologic findings that include lymphadenopathy, consolidation, pleural effusion, and miliary nodules. Radiology reports should describe whether the radiograph shows entirely normal findings, shows infiltrate, calcified granulomas, shows fibronodular scarring (noting the duration of stability), or shows findings that raise concern for active tuberculosis(11).

In both TB groups it was noted that microbiology examination testing showed the highest number was a negative result. Other studies say that children exposed to smear (+) 60% -80% are infected with TB(12). The age distribution of children who have TB in both groups shows that in the distribution of data about the age of the child has shown a homogeneous age. As said in previous studies that children aged <5 years have a higher percentage than age > 5 years(12). Children aged <5 years have a greater risk of progressing infection into TB disease due to imperfect cellular immunity(13). While, another research said that the majority of the cases of definite TB in this study were amongst the 10–15 year olds (58.3%)(14).

For the age of parents in both groups also showed homogeneity. This shows that there is no difference between the two TB groups related to the age of the parents. External factors related to the age of the parents become a factor that does not generally affect the incidence of TB in both groups.
**Conclusion**

The most significant determinant factor in active TB and latent TB in children is the appearance of chest X-ray, whereas active TB shows filtration and normal appearance in latent TB.

Additional Informations

**Funding:** Authors

**Conflict of Interest:** No

**Ethical Clearance:** Yes

**References**

Musculoskeletal Disorders Complaints by Part Body
Fishermen Village Labuang Namrole South Buru District

Masudin Sangaji1, Kuswandi Saalu2, Sahrir Sillehu2, M. Taufan Umasugi2, Johanis Hursepuny2, Tri Niswati Utami3, Suparji4

1Researcher, Faculty of Fisheries and Marine Sciences, Universitas Pattimura, 2Researcher, STIKes Maluku Husada, 3Researcher, Public Health Faculty, Universitas Islam Negeri Sumatera Utara, 4Researcher, Poltekkes Kemenkes Surabaya

Abstract

Indonesian people living in coastal areas work as fishermen. Musculoskeletal disorders are felt by fishermen in the muscles and bones resulting in the decreased system of movement. The purpose of the study was to identify differences in musculoskeletal complaints based on body parts. The study design uses observational analytic, using a cross-sectional design. Fishermen’s research samples amounted to 35 people. The results of the majority of respondents aged 51-55 years and 61-65 years, tenure of > 10 years and unnatural work postures. Nordic Body Map (NBM) measurement, musculoskeletal complaints due to unnatural/forced work postures experienced by respondents. Low extremity musculoskeletal complaints with a mean value of 18.8 and the lowest score of 12 while the highest is a score of 20. Conclusions based on the Nordic Body Map (NBM) musculoskeletal complaints occur because of unnatural/forced work postures. The majority of complaints experienced are found in the lower extremities. It is recommended that fishermen dynamically adjust their work positions.

Keywords: musculoskeletal complaints, nordic body map, muscles, work posture

Introduction

Indonesia is an archipelago consisting of 17,504 islands and 2/3 of them are sea areas, where most of the coastal population has livelihoods as fishermen. Fishermen are people whose whole or part of their livelihood depends on fishing. The sea is a natural resource that is used for the benefit of people’s welfare.

By implementing occupational safety and health control technology, it is expected that workers will achieve physical endurance, workforce, and a high level of health. Besides that, occupational safety and health can be expected to create work comfort and high work safety. So, the elements in occupational health and safety are not focused on physical factors, but also mental, emotional and psychological factors.

The world food organization (Food and Agriculture Organization) entitled “The State of World Fisheries and Aquaculture 2014” released on March 2, 2016, reported that as many as 24,000 fishermen died at sea. The report mentioned that there were 4 factors that caused the high mortality rate of traditional fishermen and sea transportation users. The main cause of marine accidents that lead to loss of human life is purely human error (human error). Other causes are neglect by sea transportation providers and related agencies, as well as sea transportation safety equipment that is far from adequate and lack of standard work procedures. Specifically, in fishery activities, as much as 80 percent of marine accident factors are caused by human negligence.(1)

Fishermen in carrying out their activities face risks, among others in the form of safety risks at sea and the risk of uncertainty of catches that can be obtained. In carrying out their activities, sea cucumber fishermen need fishing facilities such as boats, engines, compressors, masks and frog legs. These facilities cannot be bought by mustard fishermen. Efforts to overcome this are
pursued by establishing a working relationship with the civil servants. This condition then becomes the starting point for the establishment of a working relationship between Ponggawa and mustard in the sea cucumber fishery business, where they agree to establish a working relationship because each has a goal to be achieved through the relationship.

Fishermen are those who have a livelihood by utilizing marine resources such as fish and other marine biotics (except seaweed) that contain the economic value (can be consumed and marketed) both continuously and seasonally, using facilities such as boats and equipment. Fishing gear (Lampe 1989: 1). Meanwhile, according to the Director-General of Fisheries, fishermen are a group of people whose lives depend directly on sea products, either by catching or cultivating. They generally live on the beach, a residential neighborhood close to the location of their activities. The development of science and technology has made the industrial world compete for efficiency and increase productivity by using increasingly complex means of production. The more complex work equipment used will increase the potential for workplace accidents if not handled as well as possible. Potential or risk of danger is a condition that there is a possibility that a work accident will arise due to a hazard. Therefore handling and controlling work accidents can be done through risk management. Risk management is a management process with a view to minimizing risk or even avoiding work accidents altogether.(2)

Capture fisheries activities are full of challenges and are faced with risks and uncertainties. One form of risk and uncertainty is accidents at sea. The International Maritime Organization (IMO) in 2007 explained that an accident was an undesirable event involving death, injury, loss or damage to ships, property loss or environmental damage. FAO (2009) says that the main cause of marine accidents that result in the loss of human life is purely human error (human error). FAO (2010) estimates that around 30 million fishermen work on 4 million fishing vessels operating in the world, around 98% of these fishermen work on vessels less than 24 meters long, which for this measure is not written in international regulations.(1)

The number of global deaths is estimated by the International Labor Organization (ILO, 2007) in 1999 to be 24,000 deaths worldwide each year.(3) FAO (2010) adds an explanation of this, namely the global death rate will be higher because there are some countries that do not have statistical data or information on global death rates. Previous studies published in the journal work intensity of fishing activities on the operation of Soma Pajeko (Mini Purse Seine) in Bitung according to Handayani et al. (2015) a fishing fisherman is required to have a strong physique so that he can work productively and his catch is satisfying.(4)

Method

This type of research is analytic observational research using cross-sectional. Data collection is done once for a particular object at a certain time period (point time approach). The population is fishermen in Labuang Village, Namrole District, Buru Regency. Samples were taken by accidental sampling technique, which was found at the time of research and was willing to be a research respondent. Univariate data analysis uses the frequency distribution to describe the characteristics of respondents. The bivariate analysis uses the Kruskal Wallis test to determine differences in musculoskeletal complaints based on the part of the body being analyzed.

Findings

The following are presented characteristics of research respondents such as age, education, occupation, marital status, length of work and length of service in the following table:
Table 1. Distribution of Respondent Characteristics

<table>
<thead>
<tr>
<th>Age</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
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<tbody>
<tr>
<td>35-40</td>
<td>2</td>
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<tr>
<td>41-45</td>
<td>6</td>
<td>17.1</td>
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<td>46-50</td>
<td>5</td>
<td>14.3</td>
</tr>
<tr>
<td>51-55</td>
<td>8</td>
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<td>56-60</td>
<td>6</td>
<td>17.1</td>
</tr>
<tr>
<td>61-65</td>
<td>8</td>
<td>22.9</td>
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<thead>
<tr>
<th>Education</th>
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<tbody>
<tr>
<td>No School</td>
<td>10</td>
<td>28.6</td>
</tr>
<tr>
<td>Primary school</td>
<td>17</td>
<td>48.6</td>
</tr>
<tr>
<td>Junior High School</td>
<td>7</td>
<td>20.0</td>
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<td>Senior High School</td>
<td>1</td>
<td>2.9</td>
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<table>
<thead>
<tr>
<th>Profession</th>
<th>N</th>
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<tbody>
<tr>
<td>Tonda Fisherman</td>
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<td>100</td>
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<table>
<thead>
<tr>
<th>Marital Status</th>
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<tbody>
<tr>
<td>Marriage</td>
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<td>100</td>
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<table>
<thead>
<tr>
<th>Length of Working</th>
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<th>%</th>
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<tbody>
<tr>
<td>&gt;3 hour</td>
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<td>100</td>
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<thead>
<tr>
<th>Years of Service</th>
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<tbody>
<tr>
<td>&gt;10 years</td>
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</tbody>
</table>

Based on table 1, most fishermen aged 51-55 (22.9%) and 61-65 (22.9%). The majority of primary school education is 48.6%. All Tonda fishermen, married status, work duration > 3 hours and work period > 10 years. The results of Kruskal Wallis analysis in the following table:

Table 2 Complaints of Musculoskeletal Disorders based on Nordic Body Map on Fishermen in Labuang Village, Namrole District, South Buru Regency

<table>
<thead>
<tr>
<th>Complaints</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complaints on the neck and head</td>
<td>2</td>
<td>4</td>
<td>3.6</td>
</tr>
<tr>
<td>Complaints of upper limb</td>
<td>7</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Complaints of lower limb</td>
<td>12</td>
<td>20</td>
<td>18.8</td>
</tr>
<tr>
<td>Complaints to the truncus</td>
<td>6</td>
<td>9</td>
<td>7.6</td>
</tr>
</tbody>
</table>

Based on the Nordic Body Map (NBM) musculoskeletal complaints due to unnatural/forced work postures that were experienced by many respondents occurred at lower extremities with a mean value of 18.8 and the lowest score of 12 while the highest was 20. The least complaints were experienced namely Complaints of the neck and head with a mean value of 3.6 and the lowest score of 2 while the highest score of 4.

Discussion

1. Respondent characteristics

Complaints of muscles and bones due to a permanent
posture at work, repetitive and for a long time can be fatal if this condition is left. Complaints of perceived pain can be temporary if the burden that causes pain is stopped. But if musculoskeletal complaints are felt at work and loading is not stopped it can cause persistent pain. In general, cases of musculoskeletal disorders are often found in workers in the industry, loading, and unloading of goods and workers who exert 90% of their work activities.

According to Tawaka\(^5\), increasing age will be followed by a decrease in maximal oxygen volume, sharpness of hearing and vision, speed of discerning things, making decisions and ability to remember long-term. So that the older the age of the worker, then he will tend to behave unsafely. One form of unsafe behavior or substandard practice is in the form of violations of SOPs. The first complaint of musculoskeletal pain is felt at the age of 35 years, the age of complaints increases.\(^5\) According to Pratiwi that age is in line with unsafe behavior, the older a person is, the higher the category of unsafe behavior is carried out, and conversely, the younger the person’s age, the category of unsafe behavior carried out is lower or even included in the safe category.\(^6\)

The education level of the majority of respondents had an elementary school education. One’s education influences one’s mindset in facing the work entrusted to him. In addition, education will also affect the level of absorption of training provided in the context of carrying out work and work safety\(^7\).

Length of work all respondents work> 3 hours. The length of work is the length of time a person has gone through since pursuing work. The length of work is a period of time or the length of time the workforce works in a place\(^8\). The length of work can describe a person’s experience in mastering the area of their duties. The longer a person works in an organization, the more experienced the person will be so that his work skills are better\(^9\).

Respondents based on tenure as fishermen> 10 years. According to Setyawati\(^10\), the longer a person’s working period, the more emotional their stability tends to be so that they can work safely and avoid unsafe actions when working. This is because adjustments to the environment have been going on for a long time along with the experience gained. Experience is someone who has worked for 15 to 30 years, can be considered an experienced fisherman and can be used as a handler\(^11\). The fishermen’s fishing experience also has an impact on the fishermen’s catch. Experience factor, according to Foster\(^12\), what determines whether a person is experienced or not can be seen from the length of time or work period taken by a person so that he can understand the tasks of a job and have done the job properly.

The respondent’s work position is to work with unnatural/forced work postures. Work posture is defined as the posture or position of body parts such as arms, body, head and other body parts during work, such as sitting, standing, squatting or posture of the arms and hands when using work tools. The results of Manoppo’s research obtained a relationship between physical activity with musculoskeletal complaints with the Spearman correlation test.\(^13\)

2. Musculoskeletal complaints

Musculoskeletal disorders based on the Nordic Body Map (NBM), unnatural / forced labor postures that are mostly experienced by most respondents with complaints on Lower Extremities with a score of 20, this occurs in tonda fishermen who carry out daily activities at sea with an unusual position natural or forced to cause musculoskeletal disorders, especially the lower extremities with a static sitting position, this is experienced by fishermen when getting fish with a certain weight requires power to attract fish by using a footstool in pulling a load of fish to be lifted into the boat lounges.

The research on Tonda fishermen in the Namrole Subdistrict of South Buru Regency was supported by Budiman\(^14\) suggesting that this unnatural position occurred because of interactions between workers and work tools that were not balanced or work tools used were not in line with worker anthropometry. An unnatural work position is a work attitude that causes parts of the body to move away from their natural position. The farther the position of the body from the center of gravity, the higher the skeletal muscle complaints. Job attitudes are not natural in general because of the incompatibility of work with the ability of workers. The position of kneeling, bending or squatting usually causes pain in the lower back or in the knee if done for a long time and continuously results in serious problems in muscles and joints.\(^7\)
One of the factors that influence the occurrence of skeletal muscle disorders. The recommended weight is 23-25 kg, while according to MoH-RI the burden should not exceed the rule, namely adult men at 15-20 kg and women (16-18) at 12-15 kg. In this case, the capture fishermen make repetitive movements and lift the fish with a load of 10 tons every day within 30 days at sea so the weight of the load exceeds the threshold value. Previous studies have found physical activities such as lifting, holding loads that are classified as heavy workloads on fishermen associated with musculoskeletal complaints.\(^{(13)}\)

**Conclusion**

Most of the Tonda fishermen in conducting activities at sea have unnatural work positions. There are differences in complaints of musculoskeletal disorders based on body parts in tonda fishermen in Labuang Village, Namrole District, South Buru Regency. It is recommended to fishermen to change positions at work, to reduce musculoskeletal complaints. Workers can do physical fitness to relax muscles and body tissues.

**Conflict of Interest**-No

**Source of Funding**-Authors

**Ethical Clearance**-Yes

**References**

Factors Related to Hearing Disorder on Traditional Fishermen in Namrole Sub-District South Buru Regency

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Abstract

Namrole sub-district, South Buru regency, is one of the sub-districts in Maluku province where the majority of the people have a livelihood as fishermen. The process of fishing is done by diving using a compressor at depths exceeding 10 meters. This causes various complaints such as headaches, spasms and hearing disorder. The purpose of this study was to determine the factors associated with a hearing disorder in traditional fishermen. The research method used is quantitative descriptive. Statistical test results using the chi-square test showed that the depth of diving has a significant correlation with a hearing disorder with a value of $\alpha = 0.01$, while the period of work, duration of work, and frequency of diving do not correlate with the hearing disorder in traditional fishermen in Namrole district, South Buru Regency.

Keywords: Fishermen, Divers, Period of Work, Depth, Hearing Disorder

Introduction

Maluku Province is an archipelago that is included in 7 island provinces out of 34 provinces in Indonesia. Maluku Province has 11 city districts that are surrounded by oceans and have very promising water resources, including South Buru Regency. South Buru Regency is a district with an administrative area of 6723 Km2 with a water area of 1,603 Km2. South Buru Regency has the potential of marine natural resources which has a large contribution to regional development, especially in the fisheries and marine sector.

This is what causes many people in the South Buru district, especially Namrole district, to have a livelihood as fishermen. These fishermen are generally more focused on catching tuna because besides its sale value is higher than other types of fish, this fish can also be exported to other countries. The fishing process carried out by the fishermen is still traditional by using simple fishing gear and carried out by diving using a compressor.

Diving is done in calm weather or during extreme weather. This results in the emergence of disease and cause accidents. The average dive process is more than 2 hours with the frequency of diving above 3 times. The duration of dive frequency causes the fishermen often experience complaints in the form of headaches, spasms, ear pain, and hearing disorder. Diseases that can be caused by diving are decompression, poisoning, vertigo, hypotemia and barotrauma. (1) The factors that cause frequent accidents are the process of diving by using a compressor for a long time. Previous studies have shown that the number of accidents of fishermen using compressors is higher than not using compressors. (2) Other factors that influence the occurrence of accidents in traditional divers are the depth of diving and the duration of diving. Both of these factors can cause decompression. (3) Another disease caused by traditional diving is ear barotrauma. According to research (4), there is a significant correlation between the use of the compressor as a diver and the occurrence of ear barotrauma. Another factor that can cause barotrauma is the depth and duration of diving. (5) For this reason, research is needed to determine the correlation between the period of work, duration of work, frequency of diving and depth of diving with the hearing disorder on traditional fishermen in Namrole Sub-district, South Buru Regency.

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Methods

This research was a quantitative descriptive study that aimed to determine the factors associated with hearing disorders in traditional divers in Namrole Sub-district, South Buru Regency. This research was conducted in Namrole Sub-district, South Buru Regency for one month.

The population in this study were 50 fishermen with active diving activities in archery fish in Namrole Sub-district, South Buru Regency. The sample in this study were divers who have the qualifications and meet the inclusion criteria. The samples size studied was 35 fishermen who actively dive.

This research was carried out through several stages as follows:

1. Initial Survey

This stage aimed to find out the problems in the field.

2. Problem Statement

In this stage, the discussion was formulated based on the field and literature review conducted.

3. Distribution of Questionnaires

Questionnaires were distributed to capture information about the characteristics of respondents, duration of diving, frequency of diving and other information related to research.

4. Data Processing

In this stage, the questionnaire data processing was done.

5. Analysis

In this stage, an analysis was carried out with considering the results of research and supporting theories and also prior research which is related to this research. The analysis was performed using chi-square statistical analysis to see the correlation between the period of work, duration of work, frequency of diving and depth of diving with a hearing disorder.

Findings

Characteristics of Respondents

After calculating, a frequency distribution was obtained based on the characteristics of the respondents as shown in table 1.

<table>
<thead>
<tr>
<th>Age</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-25</td>
<td>6</td>
<td>17.1</td>
</tr>
<tr>
<td>26-30</td>
<td>14</td>
<td>40</td>
</tr>
<tr>
<td>31-35</td>
<td>5</td>
<td>14.3</td>
</tr>
<tr>
<td>36-40</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td>46-50</td>
<td>3</td>
<td>8.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Education</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary School</td>
<td>17</td>
<td>48.6</td>
</tr>
<tr>
<td>Junior High</td>
<td>14</td>
<td>40</td>
</tr>
<tr>
<td>Senior High</td>
<td>4</td>
<td>11.4</td>
</tr>
</tbody>
</table>

| Occupation | Frequency | Percentage |
|           |-----------|------------|
| Traditional Diver | 35 | 100        |

<table>
<thead>
<tr>
<th>Marital Status</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Married</td>
<td>32</td>
<td>91.4</td>
</tr>
<tr>
<td>Unmarried</td>
<td>3</td>
<td>8.6</td>
</tr>
</tbody>
</table>

From table 1 it can be seen that based on the age characteristics, the most respondents are respondents with an age interval of 26-30 years totaling 14 people (40%) and the least are respondents with an age interval of 46-50 years which amounted to 3 people (8.6%). Based on the level of education, the majority of respondents in this study had an elementary school level (SD) with a total of 17 people (48.6%) and the least were respondents with an education level of high school (SMA) with a total of 4 people (11.4%). Based on work, all respondents are 35 traditional divers (100%). Based on marital status, respondents who were married were 32 people (91.4%) and not yet married were 3 people (8.6%).

Correlation Between Period of Work, Duration of Work, Depth of Diving, and Frequency of Diving with Hearing Disorders
The correlation between the period of work, duration of work, depth of diving and frequency of diving with the hearing disorder are described in the following tables 2, 3, 4, and 5.

**Table 2. Correlation Between Period of Work with Hearing Disorders**

<table>
<thead>
<tr>
<th>Period of Work (year)</th>
<th>Hearing Disorder Complaint</th>
<th>Total</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>&gt; 6</td>
<td>0</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>&lt; 6</td>
<td>24</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>26</td>
<td>35</td>
</tr>
</tbody>
</table>

From table 2 it is known that there was no significant correlation between the period of work with the hearing disorder (p>0.05).

**Table 3. Correlation Between Duration of Work with Hearing Disorders**

<table>
<thead>
<tr>
<th>Duration of Work</th>
<th>Hearing Disorder Complaint</th>
<th>Total</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥6 hours</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>≤6 hours</td>
<td>9</td>
<td>24</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>26</td>
<td>35</td>
</tr>
</tbody>
</table>

Based on table 3, it is known that there was no significant correlation between the duration of diving with a complaint of hearing disorder (p>0.05).

**Table 4. Correlation Between Depth of Diving with Hearing Disorders**

<table>
<thead>
<tr>
<th>Depth of Diving</th>
<th>Hearing Disorder Complaint</th>
<th>Total</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;10 metres</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>&lt;10 metres</td>
<td>7</td>
<td>26</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>26</td>
<td>35</td>
</tr>
</tbody>
</table>

Based on table 4, it is known that there was a significant correlation between the depth of diving with complaints of hearing disorder (p <0.05).

**Table 5. Correlation Between Frequency of Diving with Hearing Disorders**

<table>
<thead>
<tr>
<th>Frequency of Diving</th>
<th>Hearing Disorder Complaint</th>
<th>Total</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;3x / day</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>&lt;3x / day</td>
<td>9</td>
<td>24</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>26</td>
<td>35</td>
</tr>
</tbody>
</table>
Table 5 shows that there was no correlation between the frequency of diving with complaints of hearing disorder (p > 0.05).

**Discussion**

**Characteristics of Respondents**

Based on the results of the study in table 1, most divers are 26-30 years old. According to (6), increasing age will be followed by a decrease in maximal oxygen volume, sharpness of hearing and vision, speed of distinguishing things, making decisions and the ability to remember long-term.

The education of respondents in this study was dominated by workers with primary school education. Worker education influences the knowledge or willingness of workers to carry out their work following occupational safety and health rules.

**Correlation between the period of diving with the hearing disorder**

In the research that has been done, the results show that from 35 respondents, 2 respondents are included in the category of long-time workers (have worked ≥ 6 years). Of all respondents who were old workers, none had complaints of hearing disorder. Whereas for the category of respondents as new workers there were 33 people, 9 of them had complaints of hearing disorder and 24 others did not experience the hearing disorder. The results showed a value of α = 0.39 or the absence of a significant correlation between the period of work and hearing disorder. This study is in line with research (7) and research (8) that there is no correlation between the period of work and hearing disorder.

**Correlation between duration of diving with a hearing disorder**

The duration of the dive is the length of the diver under the sea from the first down to the surface before making the next dive. Based on the results of research that has been done, it is obtained that from 35 divers who became respondents in this study, as many as 2 people included in the category of long-time workers (have worked ≥ 6 hours). Of all respondents who were old workers, none had complaints of hearing disorder. Whereas for the category of respondents as new workers there were 33 people, 9 of them had complaints of hearing disorder and 24 others did not experience the hearing disorder. The results showed a value of α = 0.39 or the absence of a significant correlation between the period of work and hearing disorder. This study is in line with research (7) and research (8) that there is no correlation between the period of work and hearing disorder.

**Correlation between the frequency of diving with a hearing disorder**

The frequency of diving is the number of times the respondent dives in a day. According to Edmonds et. al (in Ekawati, 2005), a diver who frequently dives will more often experience trauma to the repetitive pressure on the eardrum. This will cause the balance organ in the inner ear to experience tissue swelling and blockage of the Eustachian Tubes until the perforation of the tympanic can even cause the eardrum to bleed and tear. Therefore, the more often the frequency of diving is done, the more dangerous it is to the health of the divers because they will increasingly receive pressure and they must try to equate the pressure in the ear cavity with the pressure of the surrounding water. In the research that has been done, the results show that out of 35 respondents, 2 people who have a diving frequency > 3x a day, do not experience complaints of hearing disorder, while 9 people who have a diving frequency < 3x a day, experience complaints of hearing disorder. Meanwhile, 24 other people did not experience complaints of
hearing disorder. Another thing that can affect hearing is the physical condition at the time of diving. From interviews at the time of the study, information was obtained that sometimes divers forced to dive even though they felt unwell. After an analytical test using the Chi-Square statistical test, a value of $0.392 > \alpha (0.05)$ was obtained. This shows that there is no significant correlation between the frequencies of diving with the hearing disorder in traditional divers in the Namrole Sub-district, South Buru Regency. The results of this study contradict the research conducted by Fatmawati. (10) This is caused by the lack of open character of the respondents and motivation factors in carrying out the work of fishermen as a source of livelihood so that sometimes traditional fishing communities override perceived complaints. Traditional fishermen who do not use personal protective equipment such as earplugs are not good for hearing. Ear protection devices are proven effective in protecting against hearing loss. Research by Utami (2019) through his research on the use of earplugs as protectors against noise exposure, the results of research shows that workers who use ear protection equipment, due to noise exposure in the industry, do not experience hearing loss. Earplugs are useful as a barrier to entry of noise intensity. Audiometry results have a relationship between hearing loss in the right and left ear. (11)

Conclusion

Based on the results of the study it can be concluded that the variable that has a significant correlation with the hearing disorder is the diving depth variable while the period of work, duration of work, and frequency of diving do not correlate with the hearing disorder in traditional fishermen in Namrole Sub-district, South Buru Regency.

Conflict of Interest-No

Source of Funding-Authors

Ethical Clearance- Yes

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Open Court Principle in the Implementation of Courtroom Television in Indonesia and the Psychological Mind of Defendant

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Abstract
This research aims to analyze and carry out theoretical criticism related to the open court principle, in the implementation of courtroom television in Indonesia. There are differences in the implementation of courtroom television in several cases in Indonesia. As in the case of Jessica Kumalawongso, the entire trial process, including the verification process, was broadcasted live. The research method is the social-juridical method. In such events, the presumption of innocence principle and the principle of witnesses being prohibited from communicating with each other were also damaged. The implementation of courtroom television affected the defendant’s psychological condition. Thus, this research will conduct a theoretical study related to the open court principle in the implementation of courtroom television in Indonesia and its consequences.

Keywords: Open Court, Courtroom Television, Psychological, Defendant, Principle

Introduction
Screening of court proceedings via television or what is known as courtroom television shows the influence of information technology advantages in the legal world.¹ Until now, there is no definite definition of the term courtroom television, but in his book, Paul Lambert states that courtroom television is “...one of central concerns in relation to television courtroom broadcasting is that television cameras or television operators will distract the various people who are required as part of the courtroom process. This includes witnesses, the jury, judges, lawyers, and court staff.”²

Screening of criminal case trial proceedings has also taken place in America, namely courtroom television of the O.J Simpson case in 1994.³ Courtroom television is also carried out in Indonesia, in the case of a planned murder trial with cyanide coffee. The defendant was Jesica Kumala Wongso and this event reaped a lot of pros and cons. Also, a courtroom television was held for the blasphemy case against the accused Basuki Tjahaja Purnama or Ahok (ex governor of Jakarta Special Territory).

The implementation of courtroom television is inseparable from the discussion regarding the open court principle. Also, the application of courtroom television certainly has various consequences. Another interesting thing about it is the independence of judicial power in deciding a case amid the multiple public perceptions of the trial process, which is broadcasted live on television.⁴ As is known, the truth should not influence a judge to shift their views according to the public or social trust. “Judges are accountable to God, not to social trust.” The magnitude of the judges’ responsibility to God and the important role of enforcing justice in the society require the realization of judicial power freedom from various influences, especially public opinion.⁵

That’s why what is more important is how the power of the media should be used to guard the people, and the media is also expected to have a conscience (the conscience of the press).⁶ Courts in the UK also apply the open court principle. English criminal trials are held in public. A fundamental principle of the law of many

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modern societies is that justice should be done in public before an open court. An open court principle is one way to maintain public confidence. Another purpose of the open court principle is “to protect trial fairness by preventing abuse judicial authority.” This certainly affects the court process and it indirectly impacts the evidence. So, the manifestation of the criminal law procedure’s function to seek and to find material truth or to come close to material truth is null and void.

The trial process, as a process of law enforcement in the context of prosecution must be independent. It is part of the judicial power duty. The independence, after this referred to as the independence of judicial power, is a complex idea, not merely as a value, but also a useful instrument to pursue other higher values, namely the rule of law. Independence implies giving authority to a judiciary that is free from interference from any party and is also free from the influence of other powers.

The application of open court principle in the era of information technology development has shifted and may cause multi-interpretations. As a result, there is the trial by the press, which becomes a consequence of this open court principle. It has the potential to violate other principles, such as the principles of legality, the defendant’s presumption of innocence of, and the principle of where witnesses may not communicate with each other. Also, the implementation of the courtroom television is still different in each court in Indonesia. This is because the trial broadcasting permit policy is returned to each related court.

It is undeniable that openness by conducting broadcast in court is a demanded for transparency. It is an effort to maintain the integrity of judges as law enforcers, and also to control the proceedings of the judicial process. However, if this openness turns out to have consequences that damage other principles and other rights in a fair trial, including the condition where the defendants are disturbed as they receive the public’s judgement before receiving verdict, there needs to be an idea to regulate the implementation of the broadcasting process by the mass media in the judicial process.

Based on the explanation above, it becomes a crucial question for sure, how the meaning of the open court principle shifted in the era of information technology development and the consequences of applying courtroom television in Indonesia. This is the urgency of the research.

**Research Method**

The method used in this paper is a legal research method. This research will explain the shift in the meaning of the open court principle in the era of information technology development and the consequences of the implementation of courtroom television in Indonesia, so that it can find a solutive regulatory model related to courtroom television. This study uses a statutory approach, a conceptual approach, a comparative approach, and a case approach.

**Results and Discussion**

1. **Conceptual Meaning of the Open Court Principle**

The principles of law are the basics or directions (richtlijn) in the formation of positive law. Regarding that, Meuwissen explained, “From that principle, positive law obtains its legal meaning. It also contains criteria by which the quality of the law can be assessed ... the code can be understood against the background of a principle ... a principle that underlies”.

When we come to the discussion of the law principles, we also discuss the most important and the most essential elements of the rule of law. It is reasonable that the principle is interpreted as the heart of the legal regulations. Because the legal principle holds ethical requirements, the legal principle is a bridge between the legal regulations and social ideals and the ethical views of the people. So, it can be said that through this legal principle, the rule of law changes its nature and becomes part of the ethical level.

Fuller put forward an opinion to fathom whether we can talk about the existence of a legal system. The measure is mentioned in eight principles which he called the principles of legality. Fuller himself said that the eight principles he proposed were actually more than just requirements for the existence of a legal system, but instead, he gave qualifications to the legal system, as a legal system that contained an absolute morality.

If we examine, there is a contradiction between the application of the open court principle which is achieved through courtroom television, with the presumption of innocence and also how the defendant’s psychological condition is affected by the public’s judgement. The
absence of laws related to the courtroom television implementation subsequently violates the values of the other. The presence of courtroom television’s excessive reports on judicial cases in the process of the trial also has an indirect impact on judges, witnesses, and defendants. It is what puts pressure on the psychological burden on the defendant regarding the judge’s decision.

Besides contradicting the principle of the presumption of innocence, the implementation of courtroom television that has no clear boundaries will also conflict with other provisions. It is contrary to the regulations governing the protection of witnesses and victims to maintain their security, safety, and comfort in providing their statements in the court. The domino effect of courtroom television can cause law enforcement to be hampered. It may even be dangerous for the defendant and their psychological mind.

2. The Shift in the Open Court Principle Meaning in Criminal Law Procedures

Criminal law procedure has principles which accompany the act. One of them is the open court principle. Basically, in a criminal case, an open trial is the right of the defendant, namely the right to be tried in a trial that is open to the public. Everyone can see and monitor the proceedings of the trial. The law requires the trial to be conducted not only by the parties concerned but also by the public. The purpose is to create a fair trial, to avoid arbitrary or deviant hearings so that the trial process becomes a media of educational prevention. It may also give information to the general public.

The practice of courtroom television in the narrow sense is by broadcasting the proceedings of the trial directly, whether it is a translation by the press in interpreting the open court principle or not. As a principle, of course, the sentence has an intense philosophical foundation. The nature of technology in a mediating position changes from the quality of transparency in the relationship to the quality of opacity in the hermeneutical relationship.

The development of courtroom television in response to the demands for openness in the era of information technology development turned out to be a boomerang for the parties in the trial. Judges, defendants, witnesses, and victims were targeted by the community’s opinion and the media to be discussed during the trial process. Public enthusiasm about the trial’s direct broadcast became very high. These methods of press reporting can lead to “trial by press or trial by masses opinion” and because this contradicts the principle of presumption of the defendant’s innocence, where a person must be deemed as not guilty before a definitive ruling from an unauthorized public judge.

Although sometimes the coverage made by the mass media related to a case is not entirely true, with the continuous reporting through various media, it can ultimately lead to public opinion and make people believe that the defendant must be guilty. This phenomenon is also influenced by what is called the post-truth, which can turn a thousand lies into a truth. At this stage, justice begins to experience siltation. Justice only becomes a textual narrative and is separated from the context. Justice brings about simplification, which reduces various details of the value it contains, because it is covered by the post-truth reality. As an illustration, there is an artificial reality in the tangible court, in the emergence of various kinds of public opinion regarding negative opinions created by the mass media related to the defendant which affects the truth value of the community, that the defendant must be convicted.

3. Ideal Concept of the Courtroom Television Implementation in Indonesia

Openness is a human right and is a means to maintain the spirit and integrity of the judiciary. The government and all law enforcement devices have committed to carrying out justice based on the open court principle. With regard to the process of applying the open court principle in America, the United States also did not allow coverage in any form in the courtroom, but in 1994 in the O.J. Simpson case was broadcasted due to the demands from public to access the progress of the case. Finally, the court conducted courtroom television for the O.J. Simpson’s case. But, the trial process which was broadcasted live by the mass media was apparently able to lead the opinion of the public that O.J. Simpson was innocent. The public opinion was able to influence the decision of the jury who has the authority to determine someone guilty or innocent. On October 3, 1995, O.J Simpson was found not guilty.

Therefore, in the implementation of the principle to open to the public in Poland, the practice of courtroom television is common. The courtroom television project is referred to as the Re Court Project. The difference between the implementation of courtroom television in
Poland and other countries is that the witnesses who are asked for witness evidence by judges can directly see the recording of themselves live on the live broadcast appearance screen.[29] Based on the implementation of courtroom television in the United Kingdom, America, and Poland, it can be concluded that they apply strict rules and restrictions for the application of courtroom television for trial processes. These strict limits are aimed to maintain a noble court spirit, to minimize the misuse of recording results, and to protect the identity and security of all parties in the court.

Based on comparison with several countries, Indonesia must also make strict rules and limits related to the reporting and broadcasting mechanism of the trial process that is open to the public. So, the authors provide recommendations for setting courtroom television in Indonesia as follows: The strict regulation on the mechanism of courtroom television in Indonesia is expected to be able to prevent and minimize various interventions in the trial process. It is hoped to guarantee the fulfillment of the presumption of innocence principle, to protect the psychological mind of defendant from public judgement, and to protect the safety of the parties in the trial. These regulations are also aimed to create a fair trial.

Closing

Conclusions

The meaning of the open to the public trial principle underwent a change along with the development of the information technology era. At first, the meaning of ‘open to the public’ meant that the general public could attend and witness the proceedings in the courtroom. In its development, the public wishes to be able to witness the trial process anywhere, both inside and outside the courtroom, and also anytime. Then courtroom television came as an answer to the demands of openness in the trial process in the era of information technology development.

However, in its practice, the implementation of courtroom television in Indonesia, has various consequences. These consequences include broadcasting the trial process directly by the media and that the press can continuously create public opinion, which can then lead to trial by the press. It may also disturb the psychological condition of the defendant. In addition, the limitless courtroom television violates the defendant’s rights and may disturb the principle of presumption of innocence. In fact, broadcasting the trial directly which highlights the parties in the trial also endangers the security of these parties, both directly and indirectly.

Conflict of Interest : No

Ethical Clearance : Yes

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The Criminal Liability of Doctors in the Case of Malpractice in Indonesia

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Abstract

One of the Human Rights elements that must be achieved in accordance with the ideals of the Indonesian people is the Health aspect, the profession as a doctor devotes his/her knowledge to the public interests, has freedom and independence which is oriented to human values in accordance with the code of ethics. In implementing the code of medical ethics, it as much as possible avoids the occurrence of medical error. The error can occur in the diagnostic stage such as error or delay in diagnosis, not implementing the appropriate examination, using an examination method that is no longer used or does not act on the examination or observation result, etc. The risk that occur if it is not carried out thoroughly and carefully, is that it will result in a fatal error. The method used in this research is the normative juridism method, namely the addition method by holding on to the applied norms or legal rules. The result in this research is the liability’s manifestation of the doctor who performs medical malpractice as a form of criminal legal protection in Indonesia, it is basically based on intentional or unintentional mistakes or negligence. If it results in the victim’s death, it is equal to murder, and if the victim does not die it is called an act of persecution with the sanction of persecution.

Keywords: Criminal, Legal Protection, Malpractice, Doctor

Introduction

Health is element of Human Rights which must be realized in accordance with the ideals of the Indonesian people, the general welfare of national goals. To achieve the ability to live healthy for each people in order to realize an optimal degree of public health. The profession as a doctor devotes his/her knowledge to the public interests, it has freedom and independence which is oriented to human values in accordance with the code of ethics. This code of medical ethics aims to prioritize the interests and the safety of patient, to ensure that the medical profession must always be carried out with noble intention and use the right way.

In implementing the code of medical ethics, it avoids the occurrence of medical error as much as possible, namely a failure of a medical action that has been planned to be completed resulting in not as expected, an error of action or wrong planning to achieve a goal. The error that occurred will result in or potentially injure to patient, it can be in the form Adverse Event (KTD). This is very detrimental and dangerous; the patient can experience bad things and the action giver can also be exposed to Article of law violation.

Near Miss (NC) is an event resulting from carrying out an action or not taking the action that should be taken, which can injure the patient, but serious injury does not occur because of luck. This can be seen from the number of actions taken that are dangerous but can be prevented before they occur or are handled before they cause effects, for example, the patient receives a contra indication medicine but there is no drug reaction occurred, prevention of a medicine with an overdose will be given, but other staff know and cancel it before the medicine is given, and relief of a medicine with an overdose is given, it is known early and then given the antidote. Adverse Event (KTD) is an event resulting in an unexpected injury to the patient due to an action or not taking the action that should be taken, and not because of the patient’s condition.
The error can occur in the diagnostic stage,(6) such as error or delay in diagnosis, not implementing the appropriate examination, using an examination method that is no longer used or does not act on the examination or observation result;(7) In the treatment stage, such as error in treatment procedures, therapy treatment, drug use method, and delay in responding to the result of improper care checks; in the preventive stage, such as not providing prophylactic therapy as well as inadequate monitor and follow-up; or in other technical matter such as communication failure, device or other systems failure.(8)

The risk occurred in human is so vulnerable to an action. If it is not carried out carefully and thoroughly, it will result in a fatal error. The existence of medical risks, both those that have been detected previously and those that were not detected before or unexpected at all before, in the implementation of health services is only an adequate information.

Even so, in carrying out their professional duties, health workers are normatively obliged to remain cautious, to comply with the medical standard, to carry out the professional standard of expertise, and to respect the patient rights. This is done in order to obtain legal protection for them.

In this normative definition of a worker, it raises the definition that health worker who carries out the professional duties has a special legal position, it means that in carrying out their professional duties, they are exposed to high medical risk. Based on these explanations, it needs a criminal legal protection against doctor malpractice in Indonesia.

**Research Method**

This research used library research which is a research method that is carried out by reading and studying theories relevant to the subject matter. The data collected was then processed using data processing method which consists of: Normative juridism method, namely the addition method by holding on to the applied norms or legal rules. This discussion method is used in accordance with its need to produce an acceptable discussion both in juridical and scientific terms.

**Discussion**

A jargon that reads *errare humanum est* (error is humane), seems need to be contemplated. A theory of respectable minority rule which states that a doctor is not considered negligent if he/she chooses one of the many ways of treatment that is recognized by the medical world. Basically, a doctor will be brought before a court if there has been a loss to the patient. The loss arises as a result of a breach of obligation in which an agreement had been previously made. Even though the doctor’s obligations are not detailed in the therapeutic contract, but the doctor’s obligations are not covered by the medical services standard. While the medical services standard is made based on the rights and obligations of the doctor, both those regulated in the Code of ethics and those regulated in statutory regulation.

Legal protection for the implementation of the doctor’s duty and authority who has medical risk in the Criminal Code is formulated in Article 359 and 360, the action that causes others to be seriously injured or die which was done inadvertently. As for the elements of Article 359 and 360, there are the negligence element (culpa), the existence of certain form of action, and there are serious injuries or death of others, and the existence of a causal relation between the form of action with the result of that other person death.

Elements of medical risk and medical malpractice are as follow, in the medical risk is found the negligence element, while in the medical malpractice it is clear that there is a negligence element. In addition, specifically in health services, negligence is also associated with services that do not meet or below the medical services standard which in its practice also needs to be used to distinguish between medical risk and medical malpractice. If upon the patient, it has been performed procedures according to the medical services standard, but the patient ends up seriously injured or dead, this is a medical risk. Whereas for patient who has suffered serious injury or death as a result of doctor performing services below medical standard, this means that there is a medical malpractice.

In order to avoid misunderstanding about the emergence of risks that are detrimental to the patient, it is necessary to have clear and complete information by the doctor in a language that easily understood by the patient and by remembering where the communication is carried out. This is where the importance of a health interview is, so that in the end the patient is willing to give consent for the medical action to be taken by the doctor in the effort to cure his illness in a therapeutic transaction.
It means that the negligence element is very instrumental in determining whether a doctor is convicted or not and negligence in the medical field is very closely related to the implementation of doctor professional standards. Not only the negligence element in medical risk, it also means that both Article 359 and 360 of the Criminal Code cannot be applied to the doctor’s action that has medical risk, because one of the elements of Criminal Code Article 359 or 360 is not fulfilled in medical risk.

In the case of medical accident, there is a judge’s decision to adjudicate a case that in Indonesia it indeed requires that a doctor must act carefully on every action taken. But we cannot just regard it as a negligence act for something that is actually an accident. Regarding the mistake of clinical examination, it is actually also understandable because after all, as a human being a doctor cannot escape from the possibility of making a mistake.

In addition, the doctor’s action toward patient also has justification reason as mentioned in Criminal Code Article 50 and Article 51 paragraph 1. Meanwhile, for the conviction of an error that can be interpreted as liability in criminal law, it must meet 3 elements, namely: the ability to be responsible for the action, the inner connection between the actor and the action that can be intentional (dolus) or negligence (culpa), and there is no forgiving or erasing reason.

Thus, to make sure that a medical action is not against the law, the action must be performed in accordance with the medical profession standard or carried out legeartically, as reflected by: The existence of medical indication in accordance with the concrete treatment goal, performed according to the standardized medical procedures standard, fulfilled the patient’s rights regarding informed consent.

We need to realize that doctor’s medical actions sometimes indeed produce undesirable consequences both by the doctor and the patient, even though doctor has tried their best. Because almost all medical actions are essentially persecution justified by the Constitution, especially those related to acts of anesthesia and surgery. So that the possibility of the risk of injury or even death is very difficult to avoid. Based on the analysis, the criminal law adheres to the principle of “no criminal without error”. Furthermore, in Article 2 of the Criminal Code mentioned, “criminal provisions in Indonesian statutory regulation are applied to everyone who commits an offense in Indonesia”.

The formulation of this article determines that every individual who is in the Indonesian legal territory, can be held liable for criminal liability upon the mistakes he/she made. Based on that provision, the doctor profession is inseparable from the provision of the article. Moreover, a doctor in his daily work is always involved in the acts regulated in the Criminal Code. Even though the criminal law recognizes the abolition of criminal act in the health service, namely justification and forgiving reasons as well as those contained in the jurisprudence, it does not necessarily mean the justification and forgiving reasons abolish a criminal offense for the doctor profession.

One of the jurisprudences that contains justification reason and forgiving reason in health services is the jurisprudence. This jurisprudence contains “informed consent” as a criminal offense omission. However, this does not mean that the doctor profession is freed from all criminal liability, because the justification reason and forgiving reason for the doctor’s action is only in a certain exception.

Legal protection toward doctor’s legal liability can be distinguished between liability that is not related to the implementation of his/her profession and legal liability related to his/her profession. The implementation of the doctor profession in Indonesia can still be distinguished between responsibility for professional provisions, namely the Indonesian Code of Medical Ethics (KODEKI) contained in the Decree of the Minister of Health No. 434/Men.Kes/SK/X/1983 and responsibility for legal provisions covering the administrative law, the criminal law and the civil law. Action or behavior of doctor as legal subject in their performance in society can be distinguished between daily actions that are not related to his/her profession.

There are special factors in Medicine that are not found in general applicable law, for example medical accident or risk of treatment. Some basis for the omission of penalties or special error in medicine, namely: Risk of treatment, Medical accident, Non-negligent error of judgment, Volenti non fit iniura and Contributory negligence.

Criminal law in Indonesia in the implementation of certain medical act, there is always a risk inherent in the medical act (inherent risk of treatment). If the doctor performs the medical treatment carefully, with the
patient’s permission and based on the Medical Service Standard (SPM), but it turns out that the risk persists, for example if there is an unexpected allergic reaction, as well as complications that cannot be predicted beforehand (for example, there occurs amniotic water embolism during labor), then the doctor cannot be blamed.

Article 44 of Constitution on Medical Practice as an official explanation is determined as follows, “service standard” is a guideline that must be followed by doctor or dentist in carrying out medical practice. “Service facility level” is the level of service in which the standard of personnel and equipment are in accordance with the capabilities provided. Attitude and action that must be carried out by doctor are regulated in various standards. The profession has at least 3 kinds of standards, namely: Competency standard, Behavior standard, and Service standard.

Constitution No.36 of 2009 concerning Health has formulated the criminal threat, hence the criminal threat against the error or the negligence committed by doctor resulting in patient suffering from disabilities or injuries, no longer refers solely to the provision of Article 359, 360, and 361 of the Criminal Code. The threat is contained in Article 198 of Constitution No. 36 of 2009 concerning Health. The arrangement of doctor’s legal liability towards patient in the event of malpractice, the Indonesian health law does not officially mention the term Malpractice, but only mention error or negligence in carrying out the profession (listed in Articles 54 and 55).

Thus, the malpractice term is a legal term used in Articles 54 and 55 mentioned above. Error or negligence in carrying out the profession is listed in articles 54 and 55 of Constitution No.36 of 2009 concerning Health. Article 54 and 55 stated that the sanction on medical malpractice is the imposition of disciplinary action determined by the Medical Disciplinary Council to doctor who based on the judgment of the Council have committed negligence. Whereas the compensation that must be fulfilled by the doctor concerned is carried out in accordance with the applicable statutory regulations.

Criminal offenses are listed in Article 80, Article 81 and Article 82, while criminal violation is listed in Article 84. If in the relation between a doctor and a patient in a therapeutic transaction based on the agreement of both parties, there is an inadvertent or uncareful action by the doctor, so as to cause disability or death of the patient, then the consequences are regulated in criminal law.

Indonesian law gives full rights to its people to obtain justice and to obtain it, it is done by submitting application, complaint, and lawsuit. Both in civil, criminal or administrative cases. Therefore, as a legal protection for Indonesian people, they will be tried through a free and impartial judicial process, which referred to legal procedures that guarantee an objective examination by honest and fair judge. The sanction in criminal law is basically sanction in the form of torture or restriction of freedom against the perpetrator of criminal act. With the hope that after undergoing the criminal sanction, it will cause a deterrent effect on the perpetrator or there is a preventive element against others (the society).

Conclusion

Criminal legal protection against doctor malpractice in Indonesia in the Criminal Code can not be applied to the doctor’s action who has medical risk. This is because at the medical risk, there is one element that cannot be fulfilled, namely the negligence element. However, if the negligence element from a doctor’s action can be proven, then according to the Criminal Code, the doctor can be imposed on committing act that cause serious injury or loss of patient’s life. Besides that, based on the basis of doctor’s mistake omission, namely the justification reason (treatment risk) and forgiving reason (accident occurs in difficult operation). The liability’s manifestation of the doctor who performs medical malpractice is basically based on intentional or unintentional mistake or negligence. For intentional mistake, if it results in the victim’s death, it is equal with murder, and if the victim does not die it is called an act of persecution with the sanction of persecution.

Ethical Clearence: Yes

Conflict of Interest: No

Source of Funding: Universitas Muhammadiyah Malang

References


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Abstract

Background: Child nutritional status needs to be assessed accurately so that early and appropriate management can be carried out. Indonesia has launched child growth standards in 2005, known as Growth Diagrams of Indonesian Children (GDIC), while the WHO 2006 child growth standards (WHO-CGS) is currently use as standards to assess growth of Indonesian children.

Aim: This study aims to analyze differences in the interpretation of nutritional status between 2006 WHO-CGS and 2005 GDIC.

Method: This is a cross sectional study. 1162 children aged 0-60 months in five selected villages in a public health center in East Java, Indonesia were included in this study. Interpretation of growth was categorized into weight-for-age, length/height-for-age, and weight-for-length/height based on two growth assessment standards; 2006 WHO-GCS and 2005 GDIC.

Results: 2005 GDIC detected underweight, stunted, wasted, and obese children fewer than the 2006 WHO-CGS. There are significant differences in the interpretation of weight-for-age in girls aged 0-50 weeks (p<0.001) and length/height-for-age in children aged 0-60 months (p<0.001). While weight-for-length/height showed significant differences in boys aged 0-60 months (p=0.008) and aged 6-36 months (p=0.027).

Conclusion: 2005 GDIC detects fewer children with malnutrition than 2006 WHO-CGS. The significant difference on interpretation between two growth charts was particularly found in height-for-age.

Keywords: 2006 WHO child growth standards, 2005 Growth Diagrams of Indonesian Children, malnutrition, children 0-60 months

Introduction

Malnutrition is one of the important public health problems, affecting more than 900 million people worldwide. It is responsible for the highest death rates in children and has long-term physiological impacts. Malnutrition in children has been linked to poor mental development and school performance as well as behavior disorders. Children’s height for 2 years of age is the best predictor of human capital, therefore malnutrition is associated with lower human capital. Damage suffered early in life leads to permanent decline and may also affect future generations. Malnutrition prevention might bring important health, educational, and economic benefits. Chronic diseases are especially common in malnourished children who gain rapid weight gain in their growth. Cooperation of all stakeholders is needed to act to overcome malnutrition.

The prevalence of underweight children under five years old within the world in 2011 is estimated at 16% (101 million) and has shown a decrease from 1990 with 159 million. The prevalence of stunted and very stunted toddlers in the world in 2016 was 22.9% (155.8
million). But this data has shown a decrease compared to 2011 which was 26%.\(^4\) The 2018 national prevalence of underweight and stunted children is higher than the world prevalence (17.7% and 30.8%, respectively), but it shows lower prevalence than 2013 national data (19.6% and 37.2% respectively).\(^5\) Those prevalence rates were obtained from interpretations of nutritional status using the 2006 WHO child growth standards (2006 WHO-CGS).

Large number of malnutrition prevalence becomes national priority program and concern in daily clinical management. Nonetheless, several studies have concluded that the interpretation of nutritional status using the WHO child growth standards results in overestimation of malnutrition prevalence rate.\(^6\)–\(^10\) Those studies were conducted to compare interpretation between the 2006 WHO-CGS growth chart and the national growth charts of each country. This suggests that specific factors play a role to affect child growth in each country.

Indonesia has developed 2005 growth diagrams of Indonesian children (2005 GDIC), even though it has not been widely used.\(^11\) This research will analyze the different interpretations between 2006 WHO-CGS and 2005 GDIC, and also underline the importance of using national growth charts to measure nutritional status more accurately.

**Method**

This study was cross sectional study conducted on October-November 2017. Secondary data were obtained from medical record of five selected villages in the working area of Berbek community health center in East Java, Indonesia. A total of 1162 boys and girls aged 0-60 months who took part in the routine Pos Pelayanan Terpadu (posyandu)/integrated healthcare center in their respective villages on August 2017 were included in this study. Patients with congenital anomalies, chronic diseases, or patients taking long-term corticosteroids were not included in the study.

The selection of five villages from nineteen villages was chosen used simple random sampling. Children were divided into boys and girls, and each sex group were divided into three age groups; 0-5 months, 6-36 months, and 37-60 months. Anthropometric data was collected, both weight (kg) and length/height (cm). The data were plotted based on its nutritional status using two growth charts, the 2006 WHO-CGS and 2005 GDIC. The nutritional status is categorized into weight-for-age, length/height-for-age, and weight-for-length/height. The data were statistically analyzed through comparison test using the Wilcoxon Signed-rank Test. \(p<0.05\) was considered significant.

**Results**

Table 1 shows the sample characteristics of children. From a total of 1162 children under five the majority of children were male (53.4%) and their age were 6-36 months (55.5%). Measurements using the two growth charts cannot be done in all age groups because the weight-for-age diagram of 2005 GDIC is only available for children aged 0-50 weeks and weight-for-length/height diagrams of 2005 GDIC is only available for children with length/height <60 cm. Of about 1162 children, only weight-for-age of 374 children were measured using those 2 standards, and only weight-for-length/height of 1094 children were measured using those 2 standards. However, length/height-for-age can be measure in every sex and age groups. The total prevalence of underweight, stunted, wasted, and obesity among children was smaller when interpreted using 2005 GDIC growth chart.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n (%)</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>621 (53.4%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>541 (46.6%)</td>
<td></td>
</tr>
</tbody>
</table>
Table 1. Characteristics of children and prevalence of stunted, underweight, and obesity among children

<table>
<thead>
<tr>
<th>Age</th>
<th>0-5 months</th>
<th>6-36 months</th>
<th>37-60 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>152 (13.1%)</td>
<td>645 (55.5%)</td>
<td>365 (31.4%)</td>
</tr>
<tr>
<td>Height</td>
<td>-</td>
<td>58.33 ± 5.30</td>
<td>77.11 ± 9.04</td>
</tr>
<tr>
<td>Height</td>
<td>-</td>
<td>77.11 ± 9.04</td>
<td>95.79 ± 6.21</td>
</tr>
<tr>
<td>Weight</td>
<td>-</td>
<td>6.15 ± 1.25</td>
<td>10.14 ± 3.92</td>
</tr>
<tr>
<td>Weight</td>
<td>-</td>
<td>10.14 ± 3.92</td>
<td>14.34 ± 2.41</td>
</tr>
<tr>
<td>Weight</td>
<td>-</td>
<td>WHO 2006</td>
<td>GDIC 2005</td>
</tr>
<tr>
<td>Underweight</td>
<td>20 (5.34%)</td>
<td>3 (0.80%)</td>
<td></td>
</tr>
<tr>
<td>Stunted</td>
<td>346 (29.8%)</td>
<td>24 (2.06%)</td>
<td></td>
</tr>
<tr>
<td>Wasted</td>
<td>42 (3.83%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Obesity</td>
<td>91 (8.31%)</td>
<td>22 (2.01%)</td>
<td></td>
</tr>
</tbody>
</table>

Most of children have normal weight-for-age, both according to 2006 WHO-CGS or 2005 GDIC. 2005 GDIC detects fewer underweight children in both sexes and fewer obese boys than 2006 WHO-CGS. The difference of weight-for-age interpretation was significant only for girls (p<0.001). Most of children have normal weight-for-length/height, both according to 2006 WHO-CGS or 2005 GDIC. 2005 GDIC detects fewer both wasted and obese children than 2006 WHO-CGS. The difference of weight-for-length/height interpretation was significant only for boys aged 0-60 months (p=0.008) and boys 6-36 months (p=0.027).

Height interpretation based on age comparison on male toddlers 0-60 Months old, 0-5 Months old, 6-36 Months old, 37-60 Months old (p<0.000)

Discussion

Abnormalities of child growth either under-nutrition or over-nutrition are associated with morbidity and mortality as well as long term impacts in adulthood, such as cardiovascular and metabolic disease. Therefore, precise measurement of growth is important to monitor child health and prevent malnutrition.

This study shows that both growth charts mostly detect normal growth in our study participants. Prevalence of national under-five underweight, stunted, and wasted children were higher (17.7%, 30.8%, 10.2%, respectively), but obese children was fewer (8.0%) than prevalence found in this study. However, prevalence of stunted children in this study was higher than world prevalence in 2016.
stunted, wasted, and obese children than 2005 GDIC. However, significant differences were only found in weight-for-age of girl, height-for-age of all age and sex groups, weight-for-length/height of 0-60 months boys, and 3-36 months boys.

This indicates that Indonesian children are lighter and shorter than the standard population used to develop the 2006 WHO-CGS. Previous study also suggest that the nutritional status of Indonesian children according to weight-for-length/height tends to be the same as the population of American children because Indonesian children are lighter and shorter. Previous study also suggests that the nutritional status of Indonesian children according to weight-for-length/height tends to be the same as the population of American children because Indonesian children are lighter and shorter. 11

Weight-for-age interpretation was only significantly difference in 0-50 weeks girl, and weight-for-age interpretation was only significantly difference in 0-60 months boys and 3-36 months boys. This suggests that gender plays an important role in child growth. Genetic expressions that affect body weight appear to be significantly higher in girls starting at 9 months compared to boys. 14

Racial factor is thought to be associated with child growth difference between Indonesian and American populations. But the influence of race in child growth is still controversial. Despite differences in race, WHO Multicentre Growth Reference Study Group (2006) found similar child growth rate among their subjects. It is thought that environmental factor such as nutrition have more impact on child growth, because poor nutrition and infection can increase risk of stunted children. 15 Previous review also indicate that application of specific racial and ethnic groups growth chart is not recommended because child growth are more affected by environmental factors rather than genetic factors. Therefore, WHO recommends to use only one growth chart for all child populations in the world. 16

However, some studies show that the interpretation of height-for-age using WHO chart shows different result than interpretation using height-for-age national chart of some countries. 18 Racial difference in child growth is a fact but this phenomenon is caused by inequality in environmental factors such as economics, psychosocial, and cultural factors that affect minority groups. However, previous study has shown the biological influence on child growth. Hong Kong Chinese children is shorter than WHO growth standards as a result of epigenetic constrains on growth rather than environmental factors. 19 Lipid metabolism, fat distribution, and bone age are also related to race/ethnicity. African American children have less visceral and hepatic fat, lower rates of lipolysis, and lower adiponectin than white children. African American and white children bone age mature slower than Asian and Hispanic children.

A meta-analysis from 55 countries also showed that the standard deviation score, (SDS) of European children in general are +0.5 SD, while children from Saudi Arabia and India are -0.5 SD compared to the WHO 2006 chart reference population. Research by Batubara et al (2006) also shows that Indonesian children have lower SDS than the 2000 CDC chart reference population. This data shows that there are different characteristics of child growth in various regions.

The different interpretations of two growth chart in this study indicate that estimation of children malnutrition in Indonesia using 2006 WHO-CGS is exaggerated, and perhaps Indonesian children have grown in accordance with their genetic potential. This can lead to unnecessary examination and intervention which can interfere exclusive breastfeeding and cause excessive supplemental feeding. Formula milk and excessive supplementation of food can cause excess weight which will be accompanied by long-term complications in the future. It must be considered in the national policy of malnutrition management in Indonesia. However, because children’s growth is not only influenced by race or genetic factors, good environmental factors must also be considered. Interventions to correct problems that affect the nutritional status of the population such as lack of breast milk, low or high calorie food, lack of health facilities, sanitation, and poverty must be continued.

This study also has several limitations. Our study analyzed secondary data collected from integrated healthcare center, hence anthropometric measurements tend to vary in each center. Nutrient intake including breast milk and parent socioeconomic status were not evaluated. This might lead to some bias in evaluating differences in interpretation of nutritional status.

Application of growth chart that developed from Indonesian children as standard population should be considered in estimating children with malnutrition. Further study is needed to confirm whether the interpretation of child nutritional status using growth chart is consistent with their clinical condition and also to determine whether Indonesian children have grown...
according to their genetic potential.

**Conclusion**

2005 GDIC detects fewer children with malnutrition than 2006 WHO-CGS. The significant difference on interpretation between two growth charts was particularly found in height-for-age.

**Ethical Clearance**: This research is approved by ethical clearance from Universitas Airlangga

**Source of Funding**: This research is self-funded

**Conflict of Interest**: There is no conflict of interest

**References**


3. UNICEF. 2018 global nutrition report reveals malnutrition unacceptably high and affects. 2018;


New Record of the Species Synthesiomyia Nudiseta (van der Wulp, 1883) (Diptera, Muscidae) with Forensic Study from Kerbala City, Iraq

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1College of Education for Pure Sciences/ University of Kerbala, Kerbala, Iraq,
2Iraq Natural History Research Center and Museum, University of Baghdad, Baghdad, Iraq

Abstract

The present study aimed to investigate Synthesiomyia nudiseta (van der Wulp, 1883) in the carcasses of dogs and rats in four different localities of Kerbala governorate, during four seasons. The results indicated the appearance of S. nudiseta only in the spring and autumn seasons within the urban and agricultural areas, as well as the appearance of this species on the bodies of dogs only without rats. Taxonomy and morphological futures were described. S. nudiseta is belonging to the family Muscidae (Order: Diptera) is described as a first time recorded in Iraqi entomofuna. The specimens wear collected from carcasses of dogs and rats at agriculture and urban regions of Kerbala city. The diagnostic characters and mean morphological features were photoed.

Key words: Diptera, Forensic Entomology, Iraq, Kerbala City, Muscidae, Synthesiomyia.

Introduction

The species Synthesiomyia nudiseta (van der Wulp, 1883) is a wide distributed in the tropical and subtropical regions of the old and new world (1). This fly has contributed to criminal investigations for many countries such as Costa Rica, India, Malaysia, Thailand, and USA (2-9).

This species has been recorded in numerous studies on human bodies in Mexico and Peru (10). It is often recorded on human bodies in urban areas because it feeds on rubbish and decaying vegetables (11). Larval stages were found as well as the presence of adults on the body of a woman in a 13-storey building in Malaysia, the presence of its larvae determined the time of death was nine days (12). Larval stages of this species on human corpses have been studied in detail in Europe, specifically in the Institute of Forensic Medicine as well as the Spanish Legal Institute because of its importance in criminal investigations (13).

The larval stage is very important in future of criminal investigations because its presence on human bodies is evidence that the body was in indoor or urban environments (14).

In recent years, high temperatures have affected the distribution of these insects, so it is necessary to know the temperature of those areas from which the samples were taken and where they are distributed in the same area, and the association of this species with bodies as well as wounds plays an important role in determining the time of death post-mortem interval (PMI) (15). The aim of current study to investigate the presence of this species on the bodies of animals (dogs, rats) in different localities of Kerbala city.

Materials and Method

The study was during the period from 1/3/2018 to 28/2/2019 within the four seasons (spring, summer, autumn and winter). Two types of vertebrate animals were used in the study (dogs and rats) as shown in Table (1).

The animals used in the experiment were killed in two ways: the first using a sharp knife and the second by a toxic substance (Strychnine sulfate tablet), with dissolving 1 gm of the Strychnine in 5.0 ml of water and administered orally to rats at a dose of 3 ml using a medical syringe, while dogs were given the tablet directly, after killing the animals by the proves kill
methods of S. nudiseta was investigated after the corpse was placed on the ground after killed immediately to the point of complete decomposition (16).

Use three duplicates in each of the above-mentioned transactions. The insects were collected by air net and fly roll trap. The samples were taken to the laboratory and killed by freezing (24h) and so as they mounted by insect pins, the locality and date of collection were recorded. (17,18)

For identification of genus and species were using taxonomical keys: (13) (19-21).

The habitat and morphological features were taken photos by the aid of the digital microscope dino-light with scales of measurements.

Table (1) showed animals with their weights.

<table>
<thead>
<tr>
<th>Type of animal</th>
<th>Order and family</th>
<th>Scientific name</th>
<th>Wight of carcass kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dogs</td>
<td>Carnivora: Canidae</td>
<td>Canis Lupus familiaris (Linnaeus ,1758)</td>
<td>10 - 7</td>
</tr>
<tr>
<td>Rats</td>
<td>Rodentia: Muridae</td>
<td>Rattus rattus (Linnaeus ,1758)</td>
<td>0.5-0.35</td>
</tr>
</tbody>
</table>

Results and Discussion

Morphological study

In the current study, the genus Synthesiomyia and species nudiseta were registered as new record to fauna of Iraq. this genus can by recognized from closely genus by: Arista bare; prosternum setulose; scutellum with numerous setae on sides (plate: 1) it has one species (19).

**Synthesiomyia nudiseta** (van der Wulp, 1883)

Scientific classification

Order: Diptera
Suborder: Brachycera
Family: Muscidae
Subfamily: Azeliinae
Trib: Reinwaardtiini
Genus: **Synthesiomyia** Brauer & Bergenstamm, 1893: 9, 110, 178
Species: S. nudiseta
Synonyms: *Cyrtoneura nudiseta* Wulp, 1883  
**Hyadesimyia grisea** Gigliotos, 1893

**Synthesiomyia brasiliana** Brauer & vonBergenstamm, 1893

**Discretion of the species**: S. nudiseta can be diagnostic easily by the antennae and palpi are orange \ yellow in color, the last terminal segment of the abdomen is yellow.

**Body**: grey to black in color; length about 7-10 mm male and female (Figure 1 A, B)

**Head**: compound eye bare without hairs, dioptic in male but in female holoptic; antenna, pedicel with clear longitudinal fissure and has hard short bristles, flagellum yellow with arista bear; maxillary palpi yellow (Figure 2 A, B)

**Thorax**: consists of four longitudinal vittae on mesothorax, sternopleron triangular with four long bristles, the interior is longer and thicker than other.

**Legs**: black in color, the hind coxa with hairs at inner posterior margin

**Wing**: hyaline, costa expanded to R_{3+4}, subcostal not striate, apical section of vein M strongly curved forward (Figure 1B)

**Abdomen**: gray with checker board and differ from sarcophagids flies by the terminal segment is yellow.
Figure 1: (A) Adult male of *S. nudiseta*; and (B) Adult female of *S. nudiseta* with wing M1, R4+5, wing veins.

Figure 2: (A) *S. nudiseta*, with orange flagellomere (fl) and tip of abdomen (abd); and (B) head of *S. nudiseta* with flagellomere (fl) and palpe orange.

Figure (3) Level of appearance that *S. nudiseta* abut, a= autumn; s= summer
Environmental Study

The results of the study indicated the effect of attracting *S. mudiseta* insect species according to the type of animal as a number of adults were collected near the bodies of dogs, while no insect was collected near the bodies of mice may be due to the difference in the size of the body (17). The size of the body has an effect on insect attraction.

As for the method of killing the animal, it has had a great influence in attracting insects. The results of the research indicated that all the insects collected were near the dead bodies with a sharp knife, while no insect was found in the dead bodies using the poison. The amount of blood accompanying the dead body is injurious and does not exist in the case of poisoning (22).

The temperature had an effect on the appearance of *S. mudiseta*. It was observed during the research that the insect is present during the spring and autumn seasons and not fund in winter and summer. The study has indicated that the appropriate thermal range for the appearance of the insect within a temperature range of 20-30 and this is consistent with (23,24).

The results in Figure (3) indicate the geographical area of the environment has had a significant impact on the presence of the insect *S. nudiseta* as it appeared in urban and agricultural areas and does not appear at all in the desert and industrial areas. The puffiness stage is fresh (12)(24).

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** Self-funding

**References**


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